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The American Phytopathological Society

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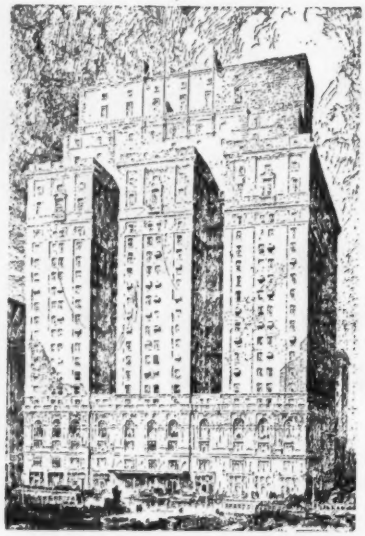

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PHYTOPATHOLOGY

VOLUME 24 NUMBER 12

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MICROORGANISMS SURVIVING THE STORAGE PERIOD OF FROZEN-PACK FRUITS AND VEGETABLES

HELEN F. SMART

(Accepted for publication March 21, 1934)

INTRODUCTION

The preservation of foods by freezing is a practice of long standing, but the practical application of freezing preservation to fresh fruits and vegetables is of rather recent development. It is believed that the earliest report in the scientific literature of practical experiments on the freezing of such products is contained in Bulletin No. 108 of the Bureau of Plant Industry, published in 1907 by S. H. Fulton (6), which describes experiments on freezing of strawberries and other small fruits. Shortly thereafter the preservation of berries in the Pacific Northwest was undertaken, and by 1928 the industry had developed from a few hundred barrels in the early years to about 100,000 barrels of 50-gallon capacity (4).

About 5 years ago small consumer packages of frozen fruit and vegetables were put on the market, and today a considerable variety of these products is available in this form. Their introduction raised the question of their healthfulness and made desirable the investigation of the microbiology of frozen-pack foods. This was undertaken by the Bureau of Plant Industry of the United States Department of Agriculture in 1929, and the present report deals with some of the findings from this work.[1]

Some 10,000 containers of frozen fruit and vegetables, (including experimental and commercial packs,) have been examined superficially for appearance, texture, taste, etc., while careful microbiological analysis has been made on not less than 3,000 samples of the available material. The data presented have been collected from frozen products packed in 50-gal. barrels, tin cans holding 5 or 10 gallons, No. 2 sealed tin cans and various kinds of glass jars and paper containers. Some of the tin cans and glass containers were sealed under vacuum.

One part of the present work has been to determine through repeated examinations of material the numbers and types of bacteria, yeasts and fungi commonly found in fruits and vegetables frozen in different ways.

[1] The writer wishes to express appreciation to Dr. C. A. Magoon, Senior Bacteriologist, Bureau of Plant Industry, under whose direction this work was done.

The subject has been treated from the viewpoint that an environment long recognized as interesting, because of a change in commercial practises, has assumed a new importance calling for exacting study. It was thought necessary also to determine the microbial flora of the fresh fruits and vegetables going into frozen-pack products, and some additions to the knowledge of ecological bacteriology have thus been made. Microorganisms growing under aerobic conditions are the only types considered in this report.

Frozen-pack material has been studied from three of the important packing centers in the country, namely, the Pacific Northwest; the eastern district centering around Norfolk, Virginia; and the southern or Louisiana district. Frozen-pack strawberries are the most widely used of any of the frozen fruits and a large part of the experimental data presented here has to do with this product. The data on strawberries will be followed by reports on other small fruits and, finally, a record of the work done on frozen pack vegetables will be given.

It has not been found feasible in all cases to determine the identity of some of the fungi and yeasts. Generic names are, at present, all that can be given in most cases. The bacteria have been identified as belonging to well-known groups but in many instances the purified cultures, when run through the prescribed tests, failed to conform in all respects to the standard reactions of species to which they obviously belonged. This failure in all probability is to be explained on the ground of disturbed metabolism resulting from the unfavorable conditions that prolonged low temperature storage of fruits and vegetables imposed or to normal variation in biochemical reactions within a bacterial species.

METHODS

One hundred grams of the material to be analyzed was measured into a sterile jar and mashed thoroughly. Sterile water to the amount of one hundred cubic centimeters was added and the mixture thoroughly agitated to bring about an equal distribution of the microorganisms. Proper dilutions were made and plates poured, using both standard beef agar at pH 7.0 and wort agar,[2] which, having a distinctly acid reaction and a higher sugar content, favors a better growth of fungi and yeasts.

The colonies of organisms on the beef-agar plates were counted at the end of 48 hours, while the wort-agar plates were incubated 4 or 5 days to allow the fungi to sporulate. The predominating types of microorganisms in all samples were noted and cultures from them made for study and identification.

[2] Wort agar formula—Hop-flavored malt syrup ..... 100 g.
Distilled water ..... 1,000 "
Agar ..... 15 "
} reaction about 4.6 pH

Every bacterial form so selected was subjected to all the standard morphological, cultural, and physiological tests prescribed by the Society of American Bacteriologists in their Manual of Methods (9). With these tests completed and using Bergey's Manual of Determinative Bacteriology (1) and Ford's Textbook of Bacteriology (5), the cultures were identified as closely as possible.

EXPERIMENTAL DATA ON STRAWBERRIES

A wide variation in the numbers of microorganisms was found on fresh strawberries depending on their condition when samples were taken. Some of the berries going into frozen packs were fresh, firm, and clean; others were over-ripe, spotted with decay, and had stood several hours before packing. Samples of the same lots of fruit after a year's storage at 15° F. showed a remarkable reduction in the number of viable organisms, amounting in some cases to practical sterilization. The fact that defrosted berries make an ideal culture medium for the growth of many microorganisms and that some undesirable and even dangerous forms may survive the freezing period make it necessary to find out exactly how many and what kinds of microorganisms are to be found in frozen packs and how they affect the quality and healthfulness of the defrosted product.

Table 1 shows the numbers of microorganisms per gram of representative samples of fresh-washed strawberries from the 3 packing centers. This table also gives the numbers of microorganisms surviving a year's storage

TABLE 1.—*Microbial content of fresh washed strawberries and of the same lots of berries after 1 and 3 years' storage in sealed tin cans and other containers at 15° F.*

Locality	Microorganisms per gram of fresh strawberries	Count per gram same lot of strawberries after 1 yr. at 15° F.	Percentage reduction in viable organisms	Count per gram same lot of strawberries after 3 yrs. at 15° F.
Northwest	14,500	525	96.3	245
"	30,500	90	99.7	32
"	1,030,000	290	99.9	50
Eastern	320,000	170	99.9	.....
"	495,000	270	99.9	.....
"	615,000	130	99.9	.....
Southern	25,500	330	98.7	.....
"	31,500	190	99.3	.....
"	67,500	170	99.7	.....
"	230,000	100	99.9	.....
Average	.....	.....	99.3	.....

of the same lots of fruit in sealed tin cans and other containers and in the case of some samples of fruit from the Pacific Northwest, 3 years' storage at freezing temperatures. The decrease in numbers of viable microorganisms is seen to be very great. There is reason to believe that rapid freezing at very low temperatures does not have the same sterilizing effect. [See Berry (3).]

The reduction in microbial content of strawberries held in sealed tin cans and other containers for a year at 15° F., as shown in this table, amounts to an average of 99.3 per cent, but the few surviving microorganisms may increase rapidly as soon as the product is defrosted, causing it to mold or ferment, depending on the type of organism gaining the ascendancy in the berries. It should be pointed out that low microbial content does not necessarily mean a high grade frozen product; other factors, such as variety of berry, method of packing, freshness of the fruit when packed, type of container, and proper control of temperature are all highly important in determining its quality.

Since the final spoilage of the frozen product is brought about by the organisms surviving the storage treatment, it was thought desirable to know what types of viable organisms remained in the frozen berries.

Table 2 gives an alphabetically arranged list of the microorganisms that were repeatedly isolated from fresh and frozen strawberries. Those occurring on the fresh fruit are shown in the first column and those surviving the freezing treatment and storage, in the second column.

This list of microorganisms isolated from fresh strawberries shows a wide range of genera, which includes, in the case of the bacteria, some that are undoubtedly of animal or human origin, such as *Aerobacter aerogenes, Staphylococcus albus, Staph. aureus,* etc. Although none of the organisms listed as isolated from the frozen fruit are known to be pathogenic, the presence of such organisms in the fresh material nevertheless suggests the need for especial care in the sanitary handling of raw material going into frozen storage.

The list of microorganisms found on fresh strawberries would undoubtedly be somewhat longer if more samples had been taken. It will be noted that some species of bacteria are reported from the frozen berries that do not appear in the list of species found on the fresh fruit. This is due in part to the fact that samples of fresh berries were taken only at intervals during the packing, while the entire pack of the frozen fruit was analyzed; and further to the fact that some types of bacteria, yeasts, and molds probably were introduced into the frozen strawberries with the sugar or syrup. Owen (7) reported the occurrence of several species of bacteria in sugar, namely *Bacillus cohaerens, B. megatherium, B. mesentericus fuscus granulatus, B. prausnitzii, B. vulgatus,* etc. It will be noted that some of these

TABLE 2.—*Microorganisms commonly found on fresh and frozen strawberries from western, eastern, and southern United States*

Fresh fruit	Frozen fruit
Bacteria	*Bacteria*
Achromobacter acidum (Conn) Bergey *et al.*	*Achromobacter acidum* (Conn) Bergey *et al.*
" *album* (Copeland) " " "	" *butyri* (Grimm) " " "
" *butyri* (Grimm) " " "	" *delmarvae* (Smart)
" *delmarvae* Smart	" *inunctum* (Pohl) " " "
Aerobacter aerogenes group	" *healii* (Buchanan & Hammer) Bergey *et al.*
Bacillus albolactis (Loëffler) Migula	" *hyalinum* (Jordan) Bergey *et al.*
" *centrosporus* Ford	" *venosum* (Vaughan) Bergey *et al.*
" *cereus* Frankland	" *viscosum* (Adametz) Bergey *et al.*
" *cohaerens* Gottheil	*Bacillus albolactis* (Loëffler) Migula
" *ellenbachensis* Stutzer	" *aterrimus* Lehmann and Neumann
" *fluorescens* Ford	" *cereus* Frankland
" *fusiformis* Gottheil	" *coagulans* Hammer
" *megatherium* de Bary	" *ellenbachensis* Stutzer
" *mycoides* Flügge	" *fluorescens* Ford
" *niger* Migula	" *fusus* Batchelor
" *parvus* Neide	" *graveolens* Gottheil
" *prausnitzii* Trevisan	" *lactis* Flügge
" *silvaticus* Neide	" *lobatus* Bergey
" *simplex* Gottheil	" *mesentericus* Trevisan
" *terminalis* Migula	" *mycoides* Flügge
" *tumescens* Zopf	" *platychoma* Gray and Thornton
Flavobacterium annulatum (Wright) Bergey *et al.*	" *polymyxa* (Prazmowski) Gruber
" *aquatilis* (Frankland) Bergey *et al.*	" *prausnitzii* Trevisan
" *butyri* Bergey *et al.*	" *ruminatus* Gottheil
" *caudatum* (Wright) Bergey *et al.*	" *silvaticus* Neide
" *flavescens* (Pohl) Bergey *et al.*	" *subtilis* (Ehrenberg) Cohn
" *flavum* (Fuhrmann) " " "	" *tumescens* Zopf
" *lutescens* (Lustig) " " "	" *vulgatus* Trevisan
Micrococcus ochraceus Rosenthal	*Micrococcus pikowskyi* Bergey *et al.*
Sarcina citrea (Migula) Bergey *et al.*	*Spirillum amyliferum* Van Tieghem
" *flava* de Bary	" *virginianum* Dimitroff
" *hamaguchiae* Saito	*Vibrio* sp.
" *lutea* Schröter	
Spirillum volutans Ehrenberg	
Staphylococcus albus Rosenbach	
" *aureus* Rosenbach	
Sphaerotilis sp.	
Proteus like *vulgaris* Hauser	
Yeasts	*Yeasts*
Saccharomyces sp.	*Saccharomyces unisporus*
Torula colliculosa	
A black yeast	
Molds	*Molds*
Alternaria sp.	*Aspergillus* sp.
Aspergillus sp.	*Cladosporium* sp.
Botrytis sp.	*Dematium* sp.
Cladosporium sp.	*Oidium* sp.
Fusarium sp.	*Monilia* sp.
Monilia sp.	*Penicillium* sp.
Mucor sp.	*Rhizopus* sp.
Oidium sp.	
Penicillium sp.	
Rhizopus sp.	
Stemphylium sp.	

species were isolated from fresh strawberries in this experiment while two of them, *B. mesentericus* and *B. vulgatus*, appear only in the frozen berries and may have been introduced with sugar or syrup.

The microorganisms listed above are the types that were repeatedly found in fresh or frozen strawberries in numbers sufficient to warrant their study. The predominant types of bacteria on both the fresh and frozen berries showed some variation in distribution, according to the locality in which the berries were grown. For instance, *Bacillus mycoides* was one of the outstanding species from all localities and appeared in great numbers on fresh strawberries and in lesser numbers in the frozen berries. *B. mycoides*, *B. fluorescens*, nearly all the commoner molds, and many varieties of yeasts were the predominating microorganisms on fresh strawberries in the west and south. The predominating type of bacteria found in all samples of fresh strawberries studied in Maryland, Delaware, and Virginia, in 1929 and 1930, was found to be a new species and was described as such at the annual meeting of American Bacteriologists at Baltimore in 1931. The name *Achromobacter delmarvae* was suggested. A condensed description of this species will be found in the Journal of Bacteriology (8). Very few rods of this species survived one year's storage in strawberries at 15° F.

The types of bacteria found in greatest numbers on frozen strawberries from a given locality remained rather constant. *Bacillus mycoides* was nearly always present, as was *B. silvaticus*, *B. cereus*, *B. mesentericus*, *B. subtilis*, and *B. platychoma*. Among the molds, Penicillium probably was the commonest type present in frozen strawberries. Several types of yeasts were found on all the plates.

A surprising number of bacterial species survived 3 years' storage in strawberries in sealed tin cans at 15° F., *Bacillus aterrimus*, *B. mycoides*, *B. lobatus*, *B. cereus*, *B. fluorescens*, and *B. subtilis* being the predominating types on most of the plates. Penicillium, both an apple-green and a blue-green species, many other molds, and many yeasts were isolated frequently from western strawberries held in sealed tin cans at 15° F. for 3 years.

Molds and yeasts were responsible for spoilage in defrosted frozen-pack strawberries. Generally a slight growth of surface mold could be seen on the strawberries in 2 or 3 days at room temperature. Following this, in most packs, there was an active fermentation caused by yeast. In many samples fermentation started without a preliminary growth of mold, and some packs showing a rather heavy surface growth of mold were not followed by gaseous fermentation.

Most other frozen small fruits showed a wide variety of bacterial species

immediately on defrosting, these being followed by the molds and yeasts that rapidly developed, and thereby destroyed the quality of the product.

EXPERIMENTAL DATA ON OTHER FROZEN PACK SMALL FRUITS

In addition to strawberries, which made up the bulk of the material studied, analyses were made of many samples of 6 kinds of other frozen-pack small fruits. Samples of the original material going into these packs were not available for study, consequently the data are only on the frozen products after storage in sealed tin cans and other containers for 1 year at 15° F. Those microorganisms appearing repeatedly on the plates in fairly large numbers were the only ones studied, which makes these lists much shorter than those of species appearing on strawberries.

Frozen blackberries, red currants, and figs had remarkably few viable microorganisms, the counts averaging about 50 per gram. Loganberries had an average count of 200 per gram, raspberries 700, and cherries 2,250 per gram when they were defrosted.

Table 3 shows a list of the bacteria, yeasts, and molds repeatedly found in the above-mentioned frozen products. It will be noted from this list that a great many nonspore-forming types of bacteria are represented as surviving the storage period at freezing temperatures, types generally thought of as being rather sensitive to extremes of temperature or other unfavorable conditions. It is true that some of the vegetative forms of bacteria have unusual powers of resistance, and it is known that very low temperatures fail to kill all microorganisms in heavily inoculated materials of various kinds. However, the presence of *Aerobacter aerogenes* and *A. cloacae* in the frozen figs emphasizes again the necessity for extra care in the preparation of these frozen foods.

Molds and yeasts were the important factors in bringing about spoilage in these fruits, as well as in strawberries. In the case of frozen-pack loganberries, the plates poured from samples immediately after defrosting showed a large number of colonies of *Bacillus mycoides* with a few colonies of *Penicillium* sp. and a smaller number of colonies of yeast. Plates poured from samples taken three days later showed *Penicillium* sp. in greatest numbers, yeasts second in importance, and a very few colonies of *B. mycoides*. Plates poured a day or two later showed almost nothing but yeasts. This sequence of growth was very striking and clear-cut in the case of loganberries. It was less so in some of the other small fruits; for instance, in cherries where a great many yeasts appeared on the plates poured from the first samples taken. It was noticed that frozen blackberries had a rather low microbial content and that it took many days for fermentation to become noticeable.

TABLE 3.—*Microorganisms commonly found in six kinds of frozen pack small fruits after storage in sealed tin cans and other containers for 1 year at 15° F.*

Frozen product	Average number microorganisms per gram	Bacteria	Yeasts	Molds
Blackberries	40	*Bacillus aterrimus* Lehmann & Neumann " *platychoma* Gray and Thornton " *mycoides* Flügge " *silvaticus* Neide " *subtilis* (Ehrenberg) Cohn *Spirillum volutans* Ehrenberg	*Saccharomyces* sp.	*Aspergillus* sp. *Penicillium* sp.
Cherries	2,250		*Saccharomyces exiguus* " *mandshuricus* " *marxianus*	*Mucor* sp. *Penicillium* sp. *Rhizopus* sp.
Figs	50 approx.	*Aerobacter aerogenes* (Kruse) Beijerinck " *cloacae* (Jordan) Bergey *et al.* *Bacillus mycoides* Flügge *Flavobacterium rigensis* Bergey *et al.* " *tremelloides* (Schottelius) Bergey *et al.* *Serratia marcescens* Bizio	*Saccharomyces* sp.	*Aspergillus* sp. *Penicillium* sp.
Loganberries	200	*Bacillus mycoides* Flügge	*Saccharomyces mandshuricus* *Torula* sp.	*Penicillium* sp.
Raspberries	700	*Bacillus albus* (Sack) Bergey *et al.* " *mycoides* Flügge " *silvaticus* Neide *Pseudomonas syncyanea* (Ehrenberg) Migula	*Saccharomyces exiguus*	*Aspergillus* sp. *Mucor* sp. *Penicillium* sp.
Red currants	60	*Bacillus albolactis* (Löeffler) Migula " *mycoides* Flügge " *silvaticus* Neide *Flavobacterium* sp. *Spirillum* sp.	*Saccharomyces* sp.	*Aspergillus* sp. *Penicillium* sp. *Rhizopus* sp.

EXPERIMENTAL DATA ON FROZEN-PACK VEGETABLES

In general it may be said that frozen vegetables present a greater problem as far as microbial growth is concerned than do frozen fruits. Vegetable materials furnish food admirably suited to bacterial growth and, since not only the spoilage of the material but the healthfulness of the product as food is involved, a study of the organisms concerned with frozen vegetable spoilage is particularly important. In this connection it may not be amiss to point out that various investigators who have considered the possibility of dangers to health from the use of frozen-pack products have indicated that, when properly prepared and handled, there is no danger to public health from their use.

The data presented here are from 8 kinds of frozen-pack vegetables from many widely separated localities.

Fresh vegetables, when washed and macerated, are known to have normally a very high microbial content, which often runs into several millions per gram of material, even for good-quality products. The defrosted vegetables showed great variation in the numbers of microorganisms per gram, but these numbers were found to be rather high in most of the samples studied. The counts varied from a few hundred to several millions per gram and were made up predominantly of bacteria, although yeasts and molds were well represented on nearly all plates.

Table 4 lists the frequently encountered forms of bacteria, yeasts, and molds that were found in frozen-pack vegetables. It will be noted that generic names are the only ones given in some instances. The reason for this is that these organisms did not seem to be similar to any of the species described in the manuals consulted and are being held for further consideration.

It will be noted in table 4 that various species of the Lactobacilli are reported from beans, peas, and corn. They have been reported by Berry (2) in peas held in storage for 26 months. The Lactobacilli seem to play an important part in the spoilage of defrosted beans, corn, peas, and perhaps of other vegetables. Yeasts also play an important rôle in the spoilage of defrosted frozen-pack vegetables, especially green beans, Lima beans, and spinach, which sometimes develop a greasy appearance as soon as they start to spoil. This greasiness, in some instances, has been found due to a species of Torula.

Beets, corn, and tomatoes are likely to show surface mold in a day or two after defrosting and later to ferment. Frozen-pack vegetables, whether blanched prior to freezing or packed unblanched, have a tendency to spoil quickly at room temperature and should be cooked before defrosting or immediately thereafter. The need for great care in preparing vegetables for frozen pack is very evident when the list of microorganisms appearing after

TABLE 4.—*Microorganisms found in frozen-pack vegetables, sealed in tin cans and other containers and stored at 15° F.*

Product	Number micro-organisms per gram	Bacteria	Yeasts	Molds
Green beans	50 to 7,000	*Bacillus mycoides* Flügge *Flavobacterium butyri* Bergey *et al.* *Lactobacillus* sp. *Pseudomonas fluorescens* Ford *Sarcina flava* de Bary " *psychrocarterica* (Rubentschick) Bergey *et al.*	*Torula* possibly *glutinus*	
Beets	68,000	*Flavobacterium flavescens* (Pohl) *Bacillus mycoides* Flügge	*Saccharomyces exiguus* Kramir's Red Torula	*Penicillium* sp.
Corn	275–24,000	*Achromobacter* sp. *Flavobacterium diffusum* (Frankland) Bergey *et al.* *Lactobacillus* sp. *Pseudomonas ovalis* (Ravenel) Chester		*Rhizopus* sp.
Lima beans	300 to over a million	*Sarcina* sp. *Streptococcus* sp.	*Saccharomyces* sp. *Torula* sp.	*Cladosporium* sp. *Dematium* sp. *Monilia* sp. *Penicillium* sp.
Mushrooms	100–36,000	*Achromobacter* sp. *Bacillus coli*-like organism (unable to identify) " *fluorescens* Ford " *mycoides* Flügge *Flavobacterium suaveolens* Soppeland *Sarcina hamaguchiae* Saito		*Aspergillus* sp. *Mucor* sp. *Penicillium* sp. *Trichoderma* sp.

TABLE 4.—*Continued*

Product	Number micro-organisms per gram	Bacteria	Yeasts	Molds
Peas	7,000 to over a million	*Achromobacter guttatum* (Zimmermann) Bergey *et al.* *Aerobacter aerogenes* (Kruse) Beijerinck *Bacillus fusiformis* Gottheil " *mycoides* Flügge *Lactobacillus* sp. *Pseudomonas aeruginosa* (Schröter) Migula *Sarcina* sp.	*Saccharomyces* sp. *Torula* sp.	*Aspergillus* sp. *Penicillium* sp.
Tomatoes	20–50	*Sarcina lactea* Bergey *et al.*	*Saccharomyces mali* *Torula* sp.	*Rhizopus* sp.
Spinach	57,000 to over a million	*Achromobacter pellucidum* Harrison *Bacillus mycoides* Flügge *Flavobacterium aurescens* (Ravenel) Bergey *et al.* *Sarcina* sp. *Streptococcus* sp.		

a year's storage at freezing temperatures is studied. The resemblance of some of the bacteria in frozen-pack products to pathogenic types suggests that great care should be taken to so label frozen-pack vegetables that they will not be mistaken for a cooked or canned product and eaten directly from the package.

SUMMARY

This paper presents the results of an investigation, extending over a period of 5 years, into the microbiology of approximately 3,000 samples of frozen-pack fruits and vegetables contained in barrels, hermetically sealed tin cans, glass jars, and paper containers, derived from the principal production centers of the United States, which may be summarized as follows:

The fresh strawberries from eastern, southern, and northwestern sources, after hulling and washing, showed a microbial content varying between the limits of a few thousands and a million or more organisms per gram of material, the content varying with the efficiency of washing, the degree of ripeness, and the soundness of the fruit.

The analysis of the frozen-pack product from the same sources after one year's storage at 15° F. in sealed tin cans and paper containers showed an average reduction in microbial content of 99.3 per cent.

No microorganisms pathogenic to human beings were isolated from the frozen-pack product, but several species of bacteria from animal or human sources were found in the fresh strawberries, thus emphasizing the need for strict sanitary control of the packing operations.

The microorganisms surviving the frozen-storage treatment in strawberries in sealed tin cans and paper containers for a year included 7 genera of fungi, a genus of yeast, and 5 genera of bacteria, the latter including approximately 30 species.

Numerous species of bacteria, and several species of molds and yeasts survived 3 years' storage at 15° F. in sealed tin cans of frozen strawberries.

Examination of other frozen small fruits including blackberries, cherries, figs, loganberries, raspberries, and red currants after storage for a year in sealed tin cans and other containers at 15° F. showed the presence of molds, yeasts, and bacteria, the numbers ranging between 50 and 2250 organisms per gram. These microorganisms were found to belong to 4 genera of molds, 2 of yeasts, and 6 genera of bacteria including about a dozen species.

Bacteria of animal or human origin were isolated from frozen-pack figs.

Microbiological examination of frozen-pack beans, beets, corn, Lima beans, mushrooms, peas, tomatoes, and spinach after storage for a year in sealed tin cans and paper containers at 15° F. showed numbers of viable organisms ranging from 20 to over a million per gram of material including

7 genera of bacteria, 2 of yeasts and 8 of molds, the higher counts being obtained from frozen products having an unknown storage history.

CONCLUSIONS

Microorganisms representing the molds, yeasts, and bacteria in considerable numbers are able to survive frozen storage in sealed tin cans and other containers of fruits and vegetables held at 15° F. for periods of 1 to 3 years, at least.

The occurrence of undesirable organisms of human or animal origin, both in the fresh material and in some of the frozen-pack products, emphasizes the need for careful sanitary control during packing operations.

BUREAU OF PLANT INDUSTRY, UNITED STATES
DEPARTMENT OF AGRICULTURE,
WASHINGTON, D. C.

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INCREASED EFFICIENCY OF CHLOROPICRIN FOR NEMATODE CONTROL WITH BETTER CONFINEMENT OF THE GAS[1]

G. H. Godfrey, Juliette Oliveira, and Helene M. Hoshino

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During the years 1930 to 1932 the nematology division of the Station conducted a series of small-scale experiments with chloropicrin for the control of the common root-knot nematode, *Heterodera marioni* (Cornu) Goodey. With the gradual improvement in technique of application, as pointed out in this paper, constantly better results were obtained. The culmination of the series in the greenhouse was a test that demonstrated the value of adequate confinement of the gas in increasing the efficiency of the treatment. The development of methods for gas confinement is presented in another paper (3). Accounts of field-plot experiments for nematode control are likewise presented elsewhere (4, 9).

CHLOROPICRIN AS A FUMIGANT

Chloropicrin, CCl_3NO_2, has been called a "tear gas" because of its high efficiency as a lachrymator, but it is completely different from the true tear gas (chloroacetophenone, $C_6H_5COCH_2Cl$), used as such generally at the present time. Pure chloropicrin is a colorless liquid, though the commercial preparation is yellowish. It is considerably heavier than water, with a specific gravity of about 1.64 at ordinary temperatures. It has an oily appearance and a sweetish, not unpleasant odor. Its vapor tension is about the same as that of water. The gas has an immediate irritating effect on the eyes, and in strong doses is irritating to the lungs and causes nausea and vomiting. Contact with the skin causes a burning that may become serious unless remedies are applied. The harmful effects of the gas in low concentrations are only temporary. High concentrations are dangerous. When these facts as to inherent possibilities of injury are thoroughly understood and ordinary precautions are taken, the chemical can be used safely by the ordinary operator, indeed much more so than can the deadly poison hydrocyanic acid gas or the highly explosive carbon bisulphide. The toxicity of chloropicrin to the lower forms of animal life, particularly to insects, is well established in the literature, most of which, up to 1930, is referred to by Roark (10) and Gersdorff (2), whose very complete bibliographies cover also the fields of the chemistry and physics of the substance and its toxicity to higher animals, including man.

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EXPERIMENTATION

The experiments described in this paper were directed primarily toward the control of the root-knot nematode. They include (1) miscellaneous preliminary laboratory tests, to establish toxicity to nematodes; (2) a series of greenhouse tests, incorporating gradually improved technique of application; and (3) small plot tests in the field.

LABORATORY TESTS

Test 1.—Heterodera marioni eggs in egg masses were killed by exposure to chloropicrin gas of unknown (but rather high) concentration in closed dishes in the laboratory during an over-night exposure of about 18 hours. Killing was proved by attempts to hatch them by methods described in another paper (8). No hatching whatever occurred, whereas control eggs hatched freely. The determination of lethal concentrations and periods of exposure remains for further experimentation.

Test 2. Studies on the Toxicity of Chloropicrin in Solution.—Chloropicrin is almost completely insoluble in water (solubility 0.14 per cent). Small drops of the chemical were placed in Syracuse dishes of water, where they rested on the bottom as distinct droplets. *Heterodera marioni* larvae were inserted. Even when they were immediately adjacent to the droplets of the chemical they remained alive and active for several hours. Ten per cent solutions of chloropicrin in 95 per cent alcohol were made up, and these diluted with water in various proportions, with a resulting faintly whitish homogeneous emulsion. Tests with these showed almost instantaneous killing of larvae at all concentrations down to 1 part of the alcoholic solution to 100 parts of water that contained 0.1 per cent chloropicrin and 0.9 per cent alcohol. This concentration of alcohol by itself is not at all lethal to nematodes. Dilutions to just half this concentration (.05 per cent chloropicrin) were not lethal, or at least not quickly so.

GREENHOUSE EXPERIMENTS

Following the preliminary laboratory tests, a number of greenhouse experiments were conducted in which the following standard technique was employed:

Heavily nematode-infested soil, was selected either directly from known badly infested fields, or from good top-soil areas, and then heavily inoculated with infested roots. The soil was next thoroughly mixed by repeated shovelings and placed in the containers in which the tests were to be conducted. The chemical was inserted, as described for each experiment. An adequate number of nontreated controls was always left for comparison. The indicator-crop method described elsewhere (5) was the basis of comparison.

Test 3.—This test, with quantities of chloropicrin ranging from 250 to 2,000 pounds per acre, was almost a complete failure for reasons not thoroughly understood but probably related to the inclusion in the soil of undecayed infested roots and to the too rapid escape of the gas. The heaviest application showed only about 60 per cent control and the others less than that.

Test 4.—In this small-scale test, wooden tubs of .65 cubic foot capacity were used as containers. Chloropicrin, at the rate of 4 cubic centimeters per tub (approximately 1000 pounds per acre), was inserted into the center of each of 4 tubs, 2 of which were covered immediately with circular pieces of tar-impregnated mulching paper, and 2 left uncovered. Seven tubs of the same soil were available as controls, for comparison. Results from this test in terms of gall counts on indicator plants, taken about 30 days after planting, were as follows:

Control tubs, gall count, average of 7, 1593 $\pm$ 69.

Chloropicrin treated, covered, (1st 40, 2nd 35); av. 37.5.

Chloropicrin treated, not covered, (1st 70, 2nd 500); av. 285.

The covered tubs showed an average of 97.7 per cent control. The uncovered varied widely, but obviously were not nearly so successful in nematode reduction as the covered. Here is an indication, then, that covering the soil makes for better confinement of the gas and consequent better control of the nematodes. This point was followed up intensively in later experiments.

Test 5. An Experiment with Coated Papers Designed to Hold the Chemical in the Soil.—The set-up of this experiment was similar to the foregoing, except that the containers were glazed stone jars of 4-gallon (15 liter) capacity. Rates of application of chloropicrin were based upon the volume of soil treated as compared with that of an acre-foot of soil. In actual practice in the pineapple field, just half the rate specified (Table 1) per acre would be used to get the same concentration of gas in the soil actually treated, in view of the fact that only alternate 3-foot strips are treated and planted. For gas confinement, the jars were covered with Kraft paper coated with a thin layer of waterglass (sodium silicate), previously applied and permitted to dry. The treated paper was cut to size and pasted tightly in place over the jar with ordinary starch paste, the edges being further sealed with an additional application of water glass. Certain jars had ordinary mulching paper, instead of the treated paper, glued over the tops as seals.

The chemical was inserted through a small hole in the cover by means of a pipette, to a depth of about 3 inches, after which the hole was immediately sealed with a sticker, which was covered finally with waterglass.

Because of the tendency of this water-glass sizing to crack upon exposure

to alternations of high and low temperatures or of degrees of dryness of the atmosphere, an effort was made to prevent it by coating the paper with self-annealing rubber latex. This proved inadequate as was shown by the gradual development, from the second day, of a fine network of crackings. The laboratory tests heretofore referred to (3) showed that when such cracks appeared the efficiency of the sizing for holding gases was immediately greatly reduced. That such occurred in this case was shown by the fact that on the fifth day after application of the chemical, when covers were removed, the odor of the chloropicrin had entirely disappeared from all jars except two of the highest applications, which were especially well sealed.

All the treatments were in triplicate, with 6 nontreated controls, 3 with covers and 3 without. The rates of application and results in nematode control are given in table 1.

TABLE 1.—*The results in nematode control of applications of chloropicrin in 4-gallon stone jars sealed with water-glass-coated paper and tar-impregnated mulching paper*[a]

Pot number	Rate of application		Nematode gall-count		Reduction
	Per jar	Per acre	3 jars	Average	
	g.	*lbs.*			*per cent*
1 to 3	.625	250	52, 32, 142	75.3	70
4 to 6	.750	300	22, 25, 5	17.3	93.1
7 to 9	.875	350	0, 23, 10	11.0	95.6
10 to 12	1.00	400	0, 20, 87	35.7	86.0
13 to 15	.875	350	18, 35, 263	105.3	58.0
16 to 18	1.00	400	14, 52, 46	37.3	85.1
19 to 21	0 (covered)	0	156, 113, 492	254	.....
22 to 24	0 (not cov.)	0	274, 69, 119	154	.....
40 to 42	1.50	600	40, 9, 4	17.7	93
Averaging jars 41 and 42 in which odor of gas was still present after 5 days				6.5	9.4

[a] Jars 13 to 18 were covered with mulching paper instead of water-glass-coated paper.

In considering the nematode results in this table it should be noted that the covers were not highly efficient for their main purpose, that of confining the fumigant. In spite of this weakness, fairly good efficiency in nematode control is manifest, particularly when results are compared with previous tests in which covers were not used. An apparent increase in efficiency with increased rate of application is evident in the first 3 series. In the

fourth, unfortunately, the cover of the last jar, being loose allowed rapid escape of the gas. This resulted in a less efficient killing of nematodes and a consequent increase in the average gall count of the three. In 1 jar each of the third and fourth series, 100 per cent efficiency was apparently attained. In jars 13 to 18, with high applications, but with mulching paper covers, rather low average efficiency occurred. In the final jars of the entire series, in which efficient confinement of the gas was manifest by odor of chloropicrin after 5 days, 97.4 per cent control was obtained. In the light of further intensive studies on soil fumigation, it would appear that some of the survival that occurred throughout was due to nematodes present within sound roots.

Another feature of this test, not disclosed in the table, involved the introduction, before treatment, of *Heterodera marioni* egg masses and infested pineapple roots into 2 of the jars (Nos. 40 and 19) 1 treatment and 1 control. The eggs were contained within small glass cells covered with silk bolting cloth, which, attached to strings, were thrust to a point well below the surface, as were also the infested roots. At the time of removing covers these were removed and egg masses from both sources (cells and roots) were placed in drops of water on slides for observations on viability. The free eggs from the control jar showed a high percentage of hatching; those from the chloropicrin jar showed none at all. Complete efficiency was thus attained in killing the organism in free egg masses. Eggs from the exposed roots all showed a high percentage of hatching, indicating lack of efficient penetration of the gas into the interior of such roots. As suggested before, the presence of such undecayed roots undoubtedly accounts for at least a part of the survival of infestation in the treated jars. On the whole, the better nematode control in this test, as compared with that of previous tests, would appear to demonstrate the value of a gas-tight cover for the purpose of prolonging the exposure of the organisms to the fumigant.

Test 6. Chloropicrin treatments in containers sealed with glue-coated papers.—For this test, an effort was made to improve over the preceding one by the use of covers of glue-coated paper, a material that does not crack upon exposure. The glue used was a water-proof aircraft adhesive prepared according to U. S. Government formula No. 11 (11), which, in laboratory tests, had shown high efficiency in preventing the escape of chloropicrin. The series consisted of the usual heavily infested soil in 5-gallon (20 liter) iron paste buckets. Chloropicrin was inserted in alcoholic solution of such concentration that multiples of 2.5 cubic centimeters gave the desired magnitudes of application in the different jars of the series. Four different rates of application were used,—200, 250, 300, and 350 pounds per acre on the basis of soil volume as related to the acre foot.

As applied in the field, in alternate strips, just half these quantities would be used per acre. Length of exposure to the fumigant was an important feature of this test, the gas-confining covers being removed in triplicate lots for each rate at 2, 4, and 7 days after treatment.

When covers were removed at the ends of the specified periods, very high efficiency in chloropicrin retention was noted in general. The gas was exceedingly strong in the 2-day jars, only a trifle less so in the 4-day, and still quite evident in the 7-day containers. Results in nematode control are shown in table 2.

TABLE 2.—*Results of soil treatment with chloropicrin in containers covered with highly impervious glue-coated papers*

Pot number	Application per acre, pounds	Period confined under cover days	Nematode gall count Containers				Percentage reduction based on controls
			1st	2d	3d	Average	
1 to 3	200	2	43	89	96	76.0	86.5
4 to 6	200	4	18	61	16	32.0	94.3
7 to 9	200	7	0	6	49	27.0	95.2
10 to 12	250	2	56	36	486	193.0	65.6
11 to 15	250	4	155	59	227	147.0	73.8
16 to 18	250	7	17	24	4	15.0	97.3
19 to 21	300	2	5	1	10	5.3	99.1
22 to 24	300	4	0	0	12	4.0	99.3
25 to 27	300	7	0	3	1	1.3	99.8
28 to 30	350	2	15	4	0	6.3	99.0
31 to 33	350	4	2	4	7	4.3	99.2
34 to 36	350	7	0	0	1	0.3	99.95
37 to 41	None	7	678, 971, 238, 300, 620			561.0	.....

Certain irregularities and inconsistencies appear that can be explained only by irregularities in gas confinement by the covers of different jars, irregularities in root growth of indicator plants, or by the presence in some jars of large, sound roots containing protected infestation. Probably all three conditions are factors. Roots of control plants were badly rotted and gall count on them was definitely lower than would have appeared had such decay not occurred. Percentage control figures, therefore, should be higher throughout than those recorded. Statistical analysis of results from 3 pots only is hardly justified. Even without such analysis, definite trends in the results are apparent. The consistent increase in efficiency of treatments with increase in period of exposure to the confined gas is evident throughout. With the higher rates of application, 300 and 350 pounds per acre, (150 and 175 pounds as applied in the alternate-row system of treatment)

100 per cent control occurred in 6 cases out of 18 and better than 99 per cent in the balance.

SMALL FIELD-PLOT TESTS

Small field-plot tests were conducted to obtain preliminary information on the practicability of the use of soil covers, and the benefits to be derived thereby in improved nematode control. Several exploratory tests are not reported, inasmuch as they served their purpose only to the extent of teaching lessons in technique applied in later trials. They did serve to show that chloropicrin is not likely to prove highly effective as a soil fungicide in saturated soil. They likewise indicated the value of a solid frame surrounding the plot sunk to a depth of several inches, with the chemical-impervious cover sealed in place upon it thus eliminating rapid diffusion of the gas away from the soil. And, finally, verification of the results of test 6, heretofore described, were obtained in part, from the fact that 4-day confinement of the gas under a cover gave better control than 2-day, and 2-day confinement better than any shorter period, or no cover at all.

Test 7. A Plot Test with Glue-coated Covers Sealed around the Edges. This test was installed in 4 × 8 ft. galvanized-iron-enclosed, isolated plots, like those described by Byars (1) and those used by Godfrey and Hagan (7) in trap-crop studies. The test involved an effort to procure good confinement of the gas in the soil, as was done in test 6 in the greenhouse, by gluing the edges of the paper to the sides of the plot frame. The attempt was not highly successful, for in all cases the seal became loose at 1 or more points by the second day. When covers were removed in 4 days, the odor of chloropicrin did not persist. Rates of application were 400 and 300 pounds per acre. Each treated plot was paired with an adjacent plot as control, the soils in the 2 having been throughly mixed in advance, to obtain uniformity of infestation. Different pairs of plots varied widely in infestation, as shown by indicator-crop readings in the controls. After treating and subsequent aeration, cowpea seeds and tomato plants were planted in alternate rows as indicator plants. The tomatoes, becoming more quickly established, had much larger and deeper root systems than cowpeas, a fact reflected in higher gall counts per root system. For soil sterilization tests tomatoes proved much superior as indicator plants, probably because of the reduction of beneficial nodule bacteria with resulting detriment to cowpea growth. Detailed results in gall counts and in effects on plant growth, are given in table 3.

Detailed consideration of these results is necessary to ther proper interpretation. Two lots, number 1 and 2, were heavily infested, as shown by the gall counts in the controls on both tomatoes and cowpeas, whereas the other 2 lots were only lightly infested. In the former, the treatment

TABLE 3.—*Results from test 7, chloropicrin with covers sealed in place, in root-gall count and effect in plant growth. (N, nontreated; Tr. treated)*

Indicator	Rate per A. pounds	Number of plants		Gall count					Top weight of plants			
		N	Tr.	N	Tr	Difference	D/PE	Per cent reduction	N	Tr.	Difference	D/PE
Tomatoes Lot												
1	400	8	8	305 ± 28.6	.75 ± .26	− 304 ± 28.6	10.6	99.8	2.3 ± .31	5.1 ± .6	2.8 ± .68	4.1
2	400	8	8	607 ± 90.0	94 ± 36	− 513 ± 96	5.3	85	6.0 ± 1.4	12.1 ± 2.0	6.1 ± 1.2	5.0
3	400	9	6	11.8 ± 2.8	3.6 ± 1.9	− 8.2 ± 3.4	2.42	70	33.9 ± 2.8	26.3 ± 3.8	−7.6 ± 4.7	1.6
4	300	7	7	60.0 ± 12.9	.28 ± .18	− 59.7 ± 13	4.63	99.5	22.6 ± 2.8	13.8 ± 2.4	−8.8 ± 3.7	2.4
Cowpeas Lot												
1	400	45	24	67 ± 5.7	2.8 ± .57	−64.2 ± 5.7	11.2	95.8	Size differences in cowpeas, irregular and not significant.			
2	400	35	34	126 ± 22.8	6.4 ± 1.5	− 120 ± 23	5.2	95.1				
3	400	40	47	15 ± 1.7	.34 ± .07	−14.66 ± 1.7	8.6	97.8				
4	300	40	36	31 ± 16.2	.64 ± .18	−30.36 ± 16.2	1.9	97.9				

brought about striking reduction in nematodes, and this was reflected in significant increase in tomato plant growth, even in the short period of 30 days. With the cowpeas, while comparable reduction in nematode infection occurred, corresponding increase in plant growth did not occur, probably because of an evident simultaneous killing of the beneficial legume bacteria. In the low infestation lots the nematodes were not sufficient in numbers to retard plant growth materially, even in the controls; so such reduction as occurred from the treatments did not manifest itself in the growth of the plants.

Examination of indicators, plant by plant, disclosed somewhat erratic nematode control, indicating lack of satisfactorily uniform distribution of the chemical through the soil. In lot 2, for example, 2 tomato plants near the end of the plot showed 246 and 443 galls, respectively, the other 6 averaging only 10. These 2 plants, then, are responsible for the rather high average that had to be recorded for the treatment. It is very possible that the unreliable applicator failed to inject into the region in which they were set. Without the 2, that is, for the remainder of the plot, control was 98.4 per cent. The same irregularity was manifest in the cowpeas in the same region, 3 plants showing a high gall count, the other 31 plants a very low one. A further contributing factor to lack of high efficiency in control was undoubtedly the failure of the seal after a few hours.

Test 8. Chloropicrin in a Home-garden Plot Confined with a Gas-impervious Paper Cover Sealed to a Board Frame Border.—A final experiment in this series of small plot tests was conducted in a home-garden plot heavily infested with nematodes. Flowers of various kinds, including particularly carnations, *Dianthus caryophyllus* L., and *Calendula officinalis* L. were failing completely. The ground selected for treatment comprised an area of approximately 10 by 20 feet, inclosed within a 12-inch board border sunk to a depth of 10 inches. The soil was in fine tilth and fertile, having received a recent addition of well-rotted stable manure. A parallel area of equal size was reserved for nontreated control plot. Chloropicrin was applied at a heavy rate—800 pounds per acre—in holes about 6 inches deep, spaced 12 inches apart. The plot was immediately covered with a large sheet of glue-coated paper, water-proofed with a coating of tar paint, which was glued to the frame border. The cover was left on for 5 days. Unfortunately, at some time during this period the paper became broken, with a hole about the size of a man's foot. When the cover was removed the odor of chloropicrin was no longer apparent. The desired efficiency in gas confinement was not fully attained. An immediate effect of the treatment, however, was evident in the finding of many dead insects, particularly burrowing cockroaches (*Pycnoscelus surinamensis* L.) on the surface of the soil.

The plot was left open a day for aeration, then tomatoes and cowpeas were planted, as nematode indicators, in alternate rows at one end of the plot. Within 3 weeks after planting striking differences in favor of the treatment were evident in tomato-plant growth. At the end of 30 days alternate plants were removed for observation on root infection and for quantitative determination of differences in plant growth (Fig. 1). The results are summarized in table 4.

The outstanding result of this test was the striking increase in top weight of the tomato plants, nearly 7-fold, brought about by the treatment, after only 1 month of growth. The condition of the plants indicated that this would have been followed by equally striking yield differences, but the test was not followed through to that point. Root-gall-count differences, while significant, were not so great, on the percentage reduction basis, as in pot studies herein reported; but the lack of high percentage reduction was again brought about by a few individual plants with high gall count in the treated plot (2 of them tomatoes with 520 and 484 galls, respectively), where pockets of soil escaped the benefit of the treatment. Such

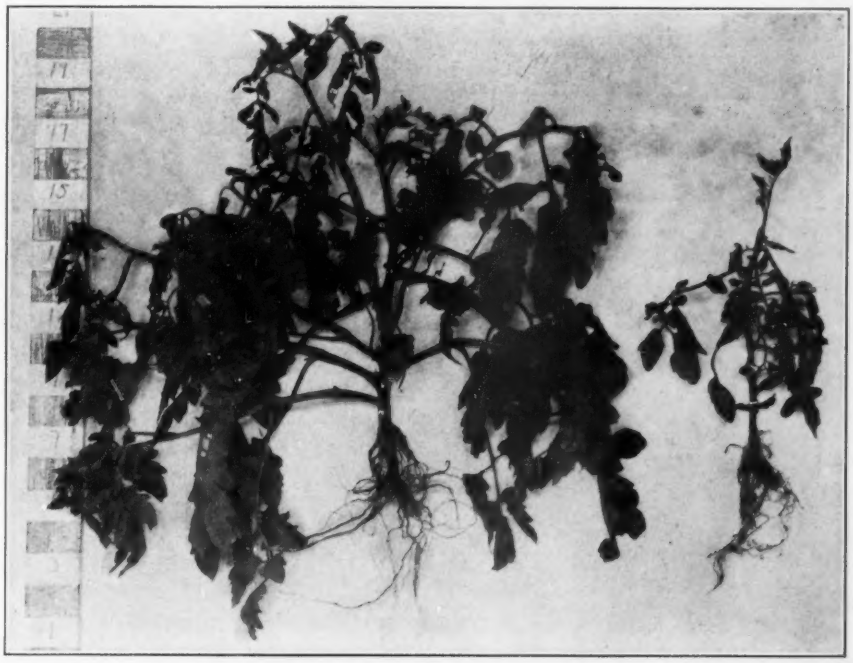

FIG. 1. Tomato plants showing the beneficial effect of a heavy soil treatment with chloropicrin, as described under Test 8 in the text. The average top weight of 11 plants from the treated plot after 30 days' growth was 7 times that of the average from the nontreated. Scale in inches.

TABLE 4.—*Results from a nematode-control experiment with chloropicrin in a small home-garden plot in Honolulu, Hawaii*

Indicator plant	Number of plants		Gall count					Top weight, grams				
	N	Tr	N	Tr	Diff.	D/PE	Percentage reduction	N	Tr	Diff.	D/PE	Percentage increase
Tomato	8	11	665.3 ± 31.2	134.7 ± 38.9	531.0 ± 50.0	10.6	80.0	20.0 ± 3.2	139.1 ± 11.3	119.1 ± 11.7	10.2	595
Cowpea	31	36	118.9 ± 12.4	13.1 ± 2.0	105.8 ± 12.5	8.5	89					

pockets either contained sound roots of the previous infested crop or were missed by the applicator. Similar escape of some of the soil under cowpea plants was also evident, 1 plant showing 116 galls and another 46, as compared with the average over all of only 13. It is believed that improved technique of application will overcome a large part of this escape from the treatment, in pockets.

FIG. 2. A secondary benefit from chloropicrin fumigation of the soil lies in its effectiveness in weed killing. Two plants of nutgrass, *Cyperus rotundus*, with the tubers cut to show the interior. Left, normal; right, completely killed, with the tuber soft and brown throughout. About $\times \frac{1}{2}$.

EFFECT OF CHLOROPICRIN ON WEEDS AND GROWING PLANTS

Another benefit from the chloropicrin treatment in this experiment, which is worthy of record, was its high efficiency in killing nut grass, *Cyperus rotundus* L., a serious weed in the Hawaiian Islands and one that is very difficult to eradicate (Fig. 2). Many plants that were growing at the time of treatment were killed outright. Examination of underground "nuts" or corms showed them to be brown throughout and decaying. After about a month a few plants appeared at the surface; these were always traceable to corms a foot or more deep in the soil. Some at a foot depth were partially discolored interiorly, but had sufficient life to bring about growth and thus to reestablish the weed in the soil. In the treated soil, however, there developed only a small fraction of the number of plants that continued to develop in the nontreated soil.

As additional evidence of the lethal effects of chloropicrin on growing plants, the results of applications to growing pineapple plants are briefly reported. In a small block of 50 plants about 6 months old, chloropicrin was inserted into the soil at the base of each plant with the Vermorel injector, at the rate of 2 cubic centimeters (3.3 grams) per plant. Within a few days the plants showed signs of injury and at the end of a month all but one were dead. Since the pineapple plant is ordinarily very hardy to unfavorable environmental conditions, this is a striking demonstration of the lethal effects of the gas on growing plants.

Another paper (6) describes the effects of low concentrations of the gas in the atmosphere on plants in the greenhouse, and emphasizes the necessity for precautions in applying treatments in enclosed spaces, such as obtain in greenhouses. It is completely impracticable to conduct a greenhouse soil fumigation with growing plants elsewhere in the same house.

DISCUSSION

Some of the greenhouse tests reported in this paper demonstrate the possibility of obtaining under ideal conditions complete control of the root-knot nematode by means of chloropicrin fumigation. None of the small plot tests was so successful. Lessons were learned from the series, however, that indicate the possibility of a high degree of efficiency when the best possible technique of application and of soil management is followed. The highest efficiency is obtainable when the following conditions are fulfilled:

(1) All roots of a previous infected crop that are left in the soil must be well rotted in order to allow efficient penetration of the gas to the points where the nematode larvae and eggs are imbedded. This requires a period of from 2 to 4 weeks or more, after the removal of the old infested crop,

under moisture, temperature, and soil-nutrient conditions favorable for decay of organic matter.

(2) The soil at the time of treatment should be in a good tilth, free from clods, and not excessively moist. (The exact moisture content that is unsafe is not known beyond the fact that treatments applied to soils that were temporarily above the "moisture equivalent" point always showed low efficiency. More precise studies in this connection are desirable.)

(3) Rates of application of 300, 350, and 400 pounds per acre (approximately 15/16 of the same figures in kilograms per hectare) have all shown good results, with efficiency in nematode control in proportion to the quantities used. The economic law of diminishing returns applies to the use of chloropicrin as it does to the use of fertilizers and other materials whose function is to increase crop yields.

(4) Points of application of chloropicrin should be not greater than 18 inches (46 centimeters) apart, and should be from 6 to 8 inches deep.

(5) The gas must be confined in the soil as efficiently as possible by means of a cover of some material that is highly impervious to it, and this cover must leave no openings that permit the free escape of gas to the open air. The more efficient the cover, the better the results are likely to be, all other conditions being equal. The cover should be maintained for at least 2 days, and preferably for 4 or more. After removal of the cover a delay of a day or 2 before planting is desirable to permit the complete escape of the free gas from the soil.

SUMMARY

Laboratory, greenhouse, and small plot tests with chloropicrin for nematode (*Heterodera marioni*) control were conducted in Hawaii during the years 1930 to 1932. In the greenhouse tests constantly improved results were obtained with improved technique of application. Adequate confinement of the gas in the soil with a cover highly impervious to the gas was found to be indispensable to best results. Ordinary Kraft paper, coated with a thin sizing of animal-product glue or casein glue, when placed over the soil and sealed at the edges, proved to be highly efficient for the purpose. Chloropicrin, applied at rates of 300 and 350 pounds per acre in closed containers, with covers as described, gave better than 99 per cent control, with 100 per cent control in several cases. The same technique applied to small field plots, with the covers sealed to metal or wood frames about the plots, did not show quite so high percentage control of nematodes but did result in remarkably increased vigor of tomato plant growth. In the treated portion of one garden plot, these plants, after only 30 days' growth, were 7 times as heavy in green top weight as the controls. Although good nematode control was manifested with cowpea-indicator plants

in the same experiment, improved plant growth did not occur because of the lethal effects of the treatment on the beneficial legume-nodule bacteria. Conditions essential to the high efficiency of chloropicrin fumigation for nematode control are (1) complete decay, before treatment, of any residue in the soil of infested roots of a previous crop; (2) good loose tilth and absence of excessive moisture; (3) rates of application of from 250 to 400 pounds per acre, depending upon efficiency desired and economic considerations and upon the efficiency of the measures for gas confinement; (4) points of application not more than 18 inches apart; (5) efficient confinement of the gas in the soil by means of a cover of gas-impervious material such as glue-coated paper, with the edges sealed to a frame or sunk well beneath the surface of the soil.

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THE HONEYBEE IN RELATION TO THE OVERWINTERING AND PRIMARY SPREAD OF THE FIRE-BLIGHT ORGANISM

A. L. Pierstorff and Howard Lamb

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The elimination of the source of the inoculum is an essential factor in fire-blight control. A number of investigators have demonstrated that the oozing hold-over canker is an important source of primary inoculum (2, 9, 3), and others. Most control measures have been based upon this observation. An additional source of primary inoculum was mentioned by Rosen (5) who stated "that the fire blight germ was isolated time and again from this material and from the bees themselves taken from the hives in early spring prior to any signs of blight in the orchard." "This material" refers to honey plus comb, pollen cells, and brood cells taken from the hives throughout the summer, winter, and early spring prior to the development of blight. He concludes, "Thus it was proved that the infected beehive is at least in part responsible for the current season's blight. . . ." The importance of this statement to both beekeepers and fruit growers gave rise to the investigation of this phase of the fire-blight problem under Ohio conditions.[1]

On April 22, 1932, 2 3-frame nucleus hives, containing brood, honey, and enough bees to care for the brood were assembled at the university apiary at Columbus, Ohio. On the same day several flasks of bouillon were inoculated by pouring into them small amounts of bouillon cultures of 2 virulent isolations of *Bacillus amylovorus* (Burr.) Trev. The following day these hives and flasks of inoculum were transported to the Flagg orchards in Scioto County, Ohio, and hive No. 1 was infested with the bouillon culture of the fire-blight organism. This inoculum was smeared over the entire contents of the hive so that the combs, frames, hive body, and bees were covered and some of the culture was poured into the hive body.

On April 25 this infested hive was placed beneath a Yellow Transparent apple tree (Tree No. 1), which had been enclosed in a bee-tight cheesecloth cage, and the bees released. The blossoms were just beginning to open. A check was prepared by tying a large cheesecloth bag securely over several branches of this tree.

On the following day hive No. 2, which had not been infested, was placed

[1] The writers wish to express their gratitude to the following fruit growers for their cooperation in this work: Mr. C. E. Drumheller manager of the Flagg orchards, Mr. Blair Adams, and Mr. Herman Mantle, and to Dr. W. E. Dunham, of the Ohio State University, who kindly provided the honey bees and apiary equipment used in these investigations.

beneath a similarly caged tree of the same variety (Tree No. 2). Just before the bees were released in this second cage, nineteen of the lowermost blossom clusters were inoculated with an atomizer containing a portion of the bouillon culture used to infest hive No. 1. A check was prepared in this cage also as described for tree No. 1. These trees were in full bloom April 28 and 29, and there was no precipitation while the bloosoms were open. Results were recorded on May 18.

On May 2 both hives were transported to the Adams fruit farm near Columbus, and the bees confined to the hives. On May 4 hive No. 1 was placed beneath tree No. 3, a cheesecloth-enclosed Grimes; hive No. 2 beneath tree No. 4 and the bees released. Full bloom occurred on May 5 and the bees were removed on May 8. When hive No. 2 was removed a handful of bees, which escaped through a small rent in the top of the cage, were clustered on the outside. Results were recorded on May 24.

An experiment similar to the one at the Flagg orchards was conducted at the Mantle and Mantle fruit farm near Painesville, in northern Ohio. Here one large "V"-shape Yellow Transparent tree was enclosed by a cheesecloth cage. A partition of the same material was placed through the center of the tree, thus dividing the cage into 2 sections with a part of the same tree in each end of the cage.

On May 13, a hive of bees was placed in each end of the cage and the bees released. On May 15, the hive in one end of the cage was infested with a virulent bouillon culture of 2 isolations of the fire-blight organism in the manner described previously for the infestation of hive No. 1 at the Flagg orchards. On the same day, a portion of this culture was atomized into 25 of the lowermost blossom clusters in the opposite end of the cage. The tree was in full bloom on May 18 and the results recorded were obtained June 1.

The data of the 1932 experiments are presented in table 1. It will be observed that no blight developed on trees 1 and 3, and we may assume that no blight bacteria were carried by the bees from the infested hives into the open blossom clusters either in the Flagg orchards or in the Adams orchard.

In tree No. 2, on which 19 blossom clusters were inoculated with blight bacteria before the bees were released inside the cage, 131 additional blossom clusters, or 66.9 per cent of the total number visited by bees developed blight. Blossom clusters on this tree protected from bee visitation by cheesecloth bags remained healthy. The results of this cage experiment indicate that fire-blight bacteria were carried from one blossom to another by honey bees.

One blighted blossom cluster was found on tree No. 4 in the Adams orchard, and may have been inoculated with bacteria carried by the bees from hive No. 2 at the Flagg orchards. This explanation appears improbable when it is noted that only one cluster out of 1165 was blighted. The

TABLE 1.—Results of 1932 cage experiments conducted to determine relation of the honeybee to the overwintering and primary spread of Bacillus amylovorus

TABLE 1.—*Results of 1932 cage experiments conducted to determine relation of the honeybee to the overwintering and primary spread of Bacillus amylovorus*

Tree No.	Location	Method of inoculation	Date bees placed in cage	Date of full bloom	Date bees removed	Date observations were taken	Healthy blossom clusters	Blighted blossom clusters	Blighted clusters
									per cent
1	Flagg orchards	Hive No. 1 placed in cage(1)	April 25	April 28–29	May 2	May 18	494	0	0.0
		Check(3)					29	0	0.0
2	Flagg orchards	Hive No. 2 placed in cage(2)	April 26	April 28–29	May 2	May 18	74	150	66.9
		Check(3)					16	0	0.0
3	Adams Fruit Farm	Hive No. 1 placed in cage(1)	May 4	May 5	May 8	May 24	745	0	0.0
4	Adams Fruit Farm	Hive No. 2 placed in	May 4	May 5	May 8	May 24	1165	1	0.085
5	Mantle orchard	Hive inoculated(4)	May 13	May 18	May 19	June 1	1364	8	0.583
5A	Mantle orchard	Tree inoculated(5)	May 13	May 18	May 19	June 1	750	790	51.3

(1) Hive No. 1 infested May 2[illegible] with bouillon culture of *B. amylovorus*.

(2) Tree No. 2 inoculated May 26 by atomizing 19 of the lowermost blossom clusters with a virulent bouillon culture of *B. amylovorus*.

(3) Checks protected from bee visitation by cheesecloth bags.

(4) Hive was infested May 15 with a bouillon culture of *B. amylovorus*.

(5) Tree 5A was inoculated May 15 by applying a virulent bouillon culture of *B. amylovorus* to 25 of the lowermost blossom clusters with an atomizer.

location of this cluster near the top of the cage and near a small rent in the cheesecloth suggests that a bee returning to the cage after visiting an adjacent Kieffer pear tree severely infected with bloosom and twig blight might have inoculated this cluster. A few bees did escape through this rent and were clustered on the outside of the cage when the hive was removed. Or, meteoric water might have inoculated this cluster by splashing bacteria from the adjacent pear tree.

In the Mantle orchard, 1364 blossom clusters on the side of the tree enclosed in the cage with the infested beehive remained healthy. Eight clusters on this side of the tree, or about 0.6 per cent of the total, were blighted. The bees may have carried the blight organism from the infested hive to the open blossom clusters, but the small amount of blight and the location of the blighted clusters near the partition suggest the possibility of inoculation by splashing rain drops carrying the blight organism. A heavy rain occurred during anthesis. Gossard and Walton (1) describe an experiment in which it appeared that the blight organism was transferred through cheesecloth by splashing rain. Miller (3) also states that splashing meteoric water is one of the most important agents concerned in the spread of fire-blight inoculum in Wisconsin. Tullis (9) draws the same conclusions for Michigan.

The spread of the blight organism to more than half of the blossom clusters on the side of the tree containing the 25 inoculated blossom clusters again demonstrates that bees carry the bacteria from blossom to blossom.

From these data it appears that, while honey bees readily carried blight bacteria from infected blossoms to others that they visited, they rarely if ever carried the organism from the hive to the blossoms.

CAGE EXPERIMENTS IN 1933

On April 10, 1933, bouillon cultures were prepared from two isolations of *Bacillus amylovorus* used in the 1932 tests. Two days later this culture was applied by means of an atomizer to the frames, combs, honey, bees, and brood in a standard hive containing the combs used in hive No. 2 at the Flagg orchards in 1932. This treatment was repeated on the following day with a similar culture. Apple twigs inoculated with a portion of this culture developed characteristic symptoms of blight.

On April 18, 2 3-frame nucleus hives were assembled at the University. The first, designated as No. 1, consisted of 3 frames of brood, honey, and bees taken from the hive inoculated April 12 and 13. Two of these frames had been in hive No. 2 at the Flagg orchards in 1932. The second nucleus hive was made up of frames containing honey, brood, and bees taken from another vigorous noninfested colony.

Hive No. 2 was infested at 6 p. m., April 19, by pouring into it 500

cubic centimeters of a mixture of the two isolations of the blight organism growing in bouillon. The hive was completely covered with this material, the combs, frames and bees being soaked, and the excess liquid was permitted to drain out of the hive. A portion of this bouillon culture injected into young growing apple twigs produced typical blight symptoms.

On April 21, these 2 infested hives were placed beneath 2 Yellow Transparent apple trees that had been enclosed by bee-tight cheesecloth cages, and the bees released. Hive No. 1 was placed beneath tree No. 1. Hive No. 2 was placed beneath tree No. 2. The bees were confined in these cages for 7 days. The trees were in full bloom on April 24 and the results were checked on May 12. On May 2 these hives were taken to the Adams Fruit Farm near Columbus and placed beneath 2 enclosed Grimes Golden apple trees, hive No. 1 being placed beneath tree No. 3 and hive No. 2 beneath tree No. 4. The bees were confined within these cages until May 8, when they were returned to the University apiary for further tests. These trees were in full bloom on May 4 and the results obtained on May 22 are presented in table 2.

It will be observed that no blight occurred in any of the caged trees used in this experiment, although the trees were enclosed in cheesecloth cages containing a beehive that had been infested with the fire-blight organism. The absence of blight in the apple trees enclosed in cages with infested hives in the tests conducted in 1932 and 1933 indicates that bees did not carry the blight organism from the hive to the apple blossoms.

The possibility of the beehive being a source of primary fire-blight inoculum in the spring is directly dependent upon the longevity of the blight organism in the hive (on the honeycomb, frames, or in the honey). That the organism may be able in some way to live over winter in or on bodies of the bees themselves appears improbable, because the bees that are active during the spring period are reared the previous autumn and would have no opportunity to come in contact with the fire-blight organism unless it were present in the hive.

It is evident from the results of the 1932 season's cage tests that the honeybee is able to disseminate the fire-blight organism from one blighted blossom cluster to another and probably from the blighted blossom clusters into the hive in the loads of nectar carried by the worker bees. Gossard and Walton (1) made a series of inoculations both before and after bloom with the mouth parts of bees collected at the entrance of the hive. These mouth parts were inserted into cuts made in young apple shoots. They found that several apparent cases of fire blight were produced in this way. They were unable, however, to recover the blight organism from the dead and dried twigs. Their results indicate that the blight organism is carried

TABLE 2.—*Results of 1933 cage experiments on determination of relation of the honeybee to overwintering and spread of Bacillus amylovorus*

Tree No.	Location	Method of inoculation	Date bees placed in cage	Date of full bloom	Date bees removed	Date observations made	Healthy blossom clusters	Blighted blossom clusters	Blighted clusters
									per cent
1	Flagg orchards	Hive No. 1 placed in cage(1)	April 21	April 24	April 28	May 12	850	0	0.0
2	Flagg orchards	Hive No. 2 placed in cage(2)	April 21	April 24	April 28	May 12	1200	0	0.0
3	Adams Fruit Farm	Hive No. 1 placed in cage(1)	May 2	May 4	May 8	May 22	680	0	0.0
4	Adams Fruit Farm	Hive No. 2 placed in cage(2)	May 2	May 4	May 8	May 22	450	0	0.0

(1) Hive No. 1 infested April 12 and 13 with a virulent bouillon culture of *B. amylovorus*.
(2) Hive No. 2 infested April 19 with a virulent bouillon culture of *B. amylovorus*.

into the hive from infected blossoms by the worker bees, and might be expected to be present in the hive during the blossoming period.

In an attempt to obtain additional information on this phase of the problem, dilution plates were prepared in September, 1932, from the honey, fragments of comb, and bits of wax taken from the frames used in the hives at the Flagg orchards. All attempts to demonstrate the presence of the fire-blight bacteria in this material resulted in failure. In April, 1933, an attempt was made to isolate the fire-blight organism from honey and fragments of wax taken from the hives used at the Mantle and Mantle fruit farm in 1932. As in the preceding case, we were unable to demonstrate the presence of *Bacillus amylovorus* in this material.

Samples of honey and comb and fragments of wax from the frame bars were taken from hives 1 and 2 on May 8, 1933, after they were returned to the university apiary. Four samples were taken from each hive with sterile instruments and placed directly into tubes of sterile bouillon. This material was allowed to incubate for 24 to 36 hours, and then inoculations were made by injecting portions of the culture into young growing apple shoots. Ten to 25 inoculations were made from each incubated sample. Checks were prepared by injecting sterile water into a similar number of apple shoots.

A further check on the presence of small numbers of the fire-blight organism was made by preparing agar dilution plates from a portion of the material used to inoculate the young apple shoots. After a sufficient growth period, transfers were made from colonies that resembled *Bacillus amylovorus* to tubes of sterile bouillon, incubated for a period of 24 to 36 hours and inoculated into young apple shoots. The presence of the fire-blight organism in the samples taken from these hives could not be demonstrated. All succeeding attempts to establish the presence of *B. amylovorus* followed the procedure just outlined.

On May 9, hive No. 2 was reinfested thoroughly with a heavy suspension of *Bacillus amylovorus* in sterile water. This suspension was applied to the entire contents of the hive with a hypodermic syringe, and directed into the empty cells of the comb, into the cells partly filled with nectar and honey, over the honey cells, and over the bees and frames. About 400 cubic centimeters of the suspension were used on the 3 frames of this hive. A portion of this inoculum produced characteristic symptoms of fire-blight when injected into young growing shoots of apple trees. Checks prepared at the same time remained healthy.

Beginning May 10, samples of honey from the open and closed cells, pieces of the comb, and bits of wax from the frame bars were collected from this hive and from hive No. 1 that was not reinfested. Four samples were collected from each hive daily until May 16, when the experiment

was terminated. These cultures were tested as previously outlined, but none of the inoculated apple shoots developed symptoms of blight. These results indicate that the blight organism probably was unable to remain viable for even 24 hours in this beehive. The results of these tests differ greatly from the findings of other workers, Gossard and Walton (1) and Thomas (8), who found the blight organism viable much longer in the honey cells and on the comb. It should be remembered, however, that their work was carried on in the laboratory, while the infested combs used in this experiment remained in the beehive.

Rosen (6) made numerous isolations from beehives located in an infected orchard. In 1929–30 isolation of *Bacillus amylovorus* from honey plus comb and from bees was reported as successful in 20 injections of young apple shoots out of a total of 2157. In 1931–33 inclusive 1277 inoculations were made with similar material and only one infection is reported. Even if the fire-blight pathogen be present in an occasional beehive, it does not follow that bees will carry this inoculum into apple blossoms and thus cause blight epiphytotics. In the cage experiments reported in tables 1 and 2 of this paper 6957 blossom clusters were exposed to inoculation by bees from hives heavily infested with *B. amylovorus*. Only 9 clusters blighted and there were other sources of inoculum for these blossoms. Even if 9 blossom clusters out of 6957 were infected from beehive material, no orchardist would consider this a serious source of inoculum.

In an attempt to determine whether the blight organism was present on the insects, bees were collected from both hives on May 8. Beginning May 9, when hive No. 2 was reinfested, bee samples were taken from this hive only.

An average of 25 bees was collected daily from the combs with sterile forceps and killed by crushing the thorax. The heads were removed and placed in tubes of sterile bouillon, 5 or 6 heads per tube. The bodies were placed in other tubes of bouillon, 3 or 4 bodies being placed in each tube. Tests for the presence of *Bacillus amylovorus* were made in the manner previously described.

The inoculated shoots were inspected 7 to 10 days after inoculation and the percentage of blight recorded. Apple shoots inoculated with bouillon, in which the heads of bees taken from both hives on May 8 were incubated, remained healthy. Two apple shoots out of 50, which were inoculated with bouillon in which heads taken from hive No. 2 on May 10 and 11 were incubated, developed blight symptoms. Inoculum prepared from this hive after May 11 gave negative results. Bouillon in which bee bodies were incubated failed to produce blight symptoms at all times.

The results of these tests indicate that the blight organism is able to

remain viable for at least 2 days on the heads of bees in an infested beehive, but it was not obtained from the honey or comb 24 hours after it was placed in the hive.

LONGEVITY OF BACILLUS AMYLOVORUS IN PURE HONEY

Gossard and Walton (1) found in 1915 that the bacteria remained viable for 47 hours in honey. In 1916 they repeated the work and found that the organism was able to survive for 72 hours in honey and perhaps longer, since the experiment was discontinued after that length of time.

Thomas (8) reports that virulent fire-blight bacteria were isolated from an infested comb at every attempt up to 55 days, when the experiment was terminated. He also was able to isolate viable fire-blight bacteria from honey in the comb 15 days after it was infested with the organism.

Pure honey in the comb was artificialy infested, in the laboratory, with the fire-blight organism as follows: (a) Transfers of *Bacillus amylovorus* were made from colonies of the organism growing on agar slants to the honey in the cells by means of a needle. The bacteria were mixed with the honey as completely as possible. Several transfers were made to each cell. (b) Other cells in the same comb were infested by injecting a bacterial suspension into the honey in these cells by means of a hypodermic needle. The needle was thrust through the cell sap and into the honey. As the needle was withdrawn, the suspension was expelled and mixed throughout the contents of the cell. Small droplets of this suspension, which oozed out of the cell through the opening made in the cap by the needle, streamed down over the cells below, thus infesting the outside of the comb.

Samples, consisting of loops of honey and bits of the cell walls and caps, were taken at 24-hour intervals for 16 days and tested for the presence of *Bacillus amylovorus*.

Apple shoots inoculated with material as described under (a), for 11 consecutive days, developed the characteristic symptoms of fire-blight. Thereafter all results were negative. Inoculations made from honey infested with the bacterial suspension as described under (b) indicate that the organism remained viable in this material for 9 days but not longer. The results of this experiment indicate that *Bacillus amylovorus* was able to survive much longer in honey or on combs in the laboratory than in the beehive.

On May 9, 3 to 4 cubic centimeters of pure honey were placed in each of 4 sterile test tubes. This honey was infested with fire-blight bacteria that were mixed thoroughly with the honey by means of the transfer needle. Two different isolations of the organism were used in this experiment, two tubes of honey being infested with each one. Transfers were made from the infested honey to bouillon each day for 7 days. All samples of this

honey taken for 5 consecutive days after infestation produced blight in each branch inoculated with it—from 10 to 20 for each test. Thereafter tests failed to give infection.

As a further check on this problem a solution of dextrose and levulose was prepared that duplicated the sugar content of apple honey as given in the "ABC and XYZ of Bee Culfure" (4). This solution consisted of 31.6 per cent dextrose and 42.00 per cent levulose. Tubes containing ten cubic centimeters of this sugar solution were infested with *Bacillus amylovorus* taken from agar-slant colonies. Samples of this material were transferred to tubes of sterile bouillon daily for 12 days and handled in the manner previously described for the tests with honey.

Samples of this solution collected the first 5 days after it was infested with the blight organism produced blight when injected into young growing apple shoots. The results of all later tests were negative. The effects of this sugar solution on the blight organism were apparently similar to the effects of pure honey, since viable organisms were not obtained from either the honey or the sugar solution after 5 days.

Of interest in this connection are numerous experiments conducted by Sackett (7). Some of his results are as follows: *Bacillus fecalis alkaligenes,* dead in 5 hours; *B. dysenteriae,* dead after 10 hours; *B. paratyphosus,* dead after 24 hours; *B. enteritidis* and *B. typhosus,* dead in 48 hours; *B. lactis aerogenes, B. proteus vulgaris* and *B. suipestifer* dead in 4 days, and *B. coli communis* dead on the 5th day.

Bacteriologists generally consider these organisms to be nonspore forming. Since the fire-blight bacillus is not known to form spores it may be considered to be in the vegetative state when it enters the hive in a load of nectar and when the nectar is converted into honey by the bees.

The results of other workers as given previously indicate that the life of *Bacillus amylovorus* in pure honey does not greatly exceed the longevity of other similar vegetative organisms in the same medium. This fact further indicates that the honey cells are not a probable source of fire-blight inoculum in the spring.

SUMMARY

Honeybees carried fire-blight bacteria from artificially inoculated blossom clusters on the lowermost branches to other blossom clusters on the same tree.

Beehives that had been infested with virulent cultures of *Bacillus amylovorus* were placed under apple trees enclosed within cheesecloth cages. Bees did not carry blight inoculum from the hive to the blossom clusters.

The transfer of bee colonies from one locality to another did not spread

the fire-blight organism, even though these colonies had been confined to trees badly infected with blossom blight.

The longevity of *B. amylovorus* in pure honey was found to vary from 5 to 11 days. The organism was recovered for 5 days from inoculated honey in test tubes, for 9 days from honey in the comb inoculated with a suspension of the organism, and for 11 days from honey in the comb that had been inoculated with fire-blight bacteria transferred from agar-slant colonies.

It was impossible to demonstrate the presence of *B. amylovorus* on the combs, frames, or in the honey in a beehive 24 hours after it was infested with a suspension of the organism in a virulent state.

B. amylovorus was obtained from the heads of honeybees taken from a hive 2 days after it was infested with a suspension of the virulent organism. Thereafter it could not be demonstrated that the blight organism was present on the insects.

The results of this investigation indicate that the beehive is not a probable source of fire-blight inoculum in the spring, under Ohio conditions.

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PERONOSPORA VICIAE AND INTERNAL PROLIFERATION IN PEA PODS[1]

WILLIAM C. SNYDER[2]

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INTRODUCTION

During the growing seasons of 1932–33 certain growers of market peas in California were concerned with the occurrence of chlorotic lesions and velvety internal proliferations in green pods of pea, *Pisum sativum* L. Externally, a yellowish blotch generally denoted the condition, while internally, a mealy coating of mycelium and oospores lined the cavity, or there was a felty proliferation of the inner pod membrane accompanied by oospores (Fig. 1). Sometimes the felty condition seemed to be unattended by superficial fungus growth, although still associated with the yellow blotches, and the oospores were found in the ovary wall tissues between the surface blemish and the membrane bearing the internal proliferation. Although other causes of a proliferation of the inner epithelial lining of pea pods are known (9), the present discussion is limited to proliferation that accompanies oospores.

Pod infection by downy mildew has been reported in the literature, but the internal proliferation of the pods that has been found associated with oospores in certain coastal districts of pea culture in California apparently has not been described. W. G. Smith (8), in England, noted *Peronospora viciae* (Berk.) de Bary, growing within the pods of garden peas and upon the seeds. Linford (5) referred to the yellowish white, porcelanous, slightly swollen areas on leaves, stems and pods in which oospores of the fungus are developed and noted an occasional systemic infection. Melhus (6) found mycelium in the seed coats of seeds from infected pods. In the spring of 1931 Ramsey (7) noted "what appears to be the oospore stage of Peronospora" on pods in a shipment of peas from Santa Maria, California. Heald (4) mentioned pod infection in Washington as a serious aspect of the disease, responsible for 60 per cent of culls in certain pickings, and found oospores in seed from such pods. The symptom of internal proliferation of the pea pod in association with oospores, is reported by Sorauer (10) in his chapter on intumescences. He observed that the inner parchment-like lining sometimes forms intumescences resembling mold in their white felty appearance, and that in one case he found in the intumescent tissue numer-

[1] Contribution from the Division of Plant Pathology, University of California, Berkeley, California.

[2] The writer gratefully acknowledges the helpful suggestions of Professors Ralph E. Smith and Max W. Gardner.

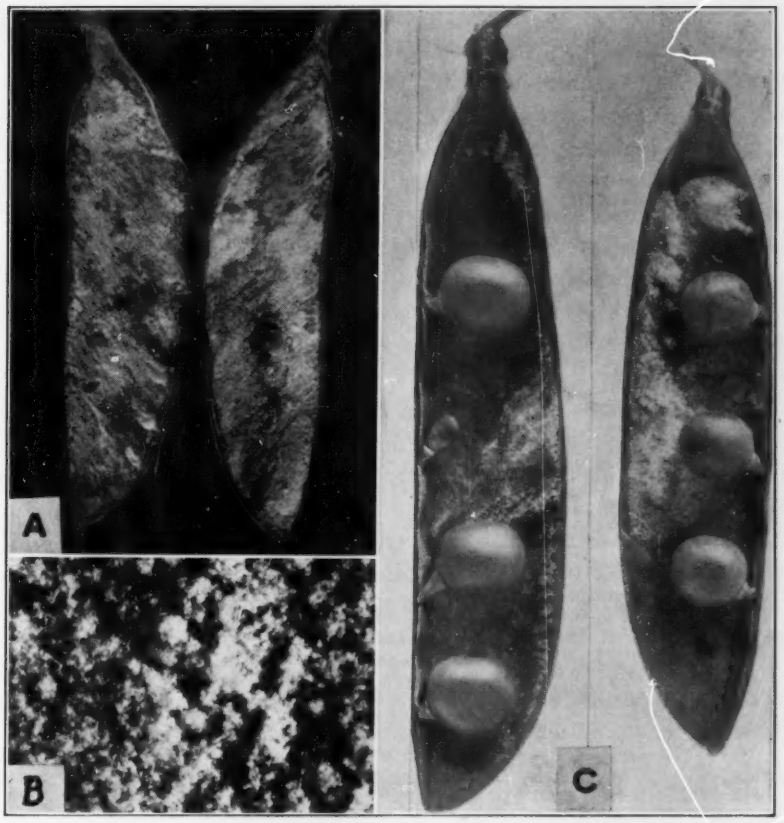

FIG. 1. A. Thin mealy layer of mycelium and oospores of *Peronospora viciae* coating the interior surface of a pea pod artificially inoculated by atomizing a plant bearing young pods, with a conidial suspension. Also typical of some natural infections in the field. B. Mycelium- and oospore-covered pod lining shown in A. ×22. C. White, felty, mold-like patches of epithelial proliferation sometimes found associated with infections of the pod wall by *Peronospora viciae*.

ous oospores that "presumably belong to *Peronospora viciae*." Sorauer attributed the cause of the intumescences, however, to environmental influences.

SYMPTOMS

The external symptoms, already briefly referred to, usually constitute the least conspicuous phase of the disease, yet it is upon the detection of these surface blemishes that packers depend in culling out pods unsuited for market. The yellowish blotches, ¼ to 1 inch or more, in extent, tend to become slightly sunken and may be homogeneous, or heterogeneous with the blotch, consisting of many small yellowish islands.

A common type of internal symptom is the presence of white, felty, mold-like patches of epithelial proliferation, occurring directly under the external yellowish blotches (Fig. 1, C). This felty layer is composed of living epithelial cells in which chloroplasts and other cell structures are visible. The delicate mat-like growth frequently involves the greater part of the membranous lining, extending $\frac{1}{8}$ inch or more into the pod cavity, and envelops the ovules. The hair-like outgrowths are several cells long, curl apically and intertwine, producing in mass the dense felty layer. Although no fungus may be evident upon either the exterior or interior surfaces of pods showing these symptoms, yet, peeling back the internal parchment-like lining, masses of oospores are found imbedded in the ovary wall directly below the velvety growth.

Where the fungus is not confined within the wall tissues, but has pushed through the inner membrane into the pod cavity, and where no proliferation occurs, the thick, nonseptate mycelium of varying diameter produces enormous numbers of oospores as it spreads over the interior surface of the pod. This thin, white coating of mycelium and oospores, which may give a mealy appearance to the entire lining of the pod cavity, resembles a dusting with flour (Fig. 1, A, B). As the oospores mature, the color of the layer changes to a cream-yellow, then brown. When such invasion takes place early in the development of the pod, the ovules generally fail to mature, and the pods are empty. Frequently, however, the ovules mature and microscopic examination has shown that the fungus may enter the seed coats.

In a combination of the proliferation of the epithelial cells and the development of the fungus in the pod cavity, the felty mat gradually assumes a brownish discoloration with the development of mature oospores scattered through it, and apparently also as a result of death of the proliferated cells evidently caused by the fungus, which may be observed twined about the hair-like growths. Here again the ovules frequently mature without any apparent abnormality.

INOCULATION TRIALS

Since there was uncertainty as to the identity of the fungus producing the oospores, because fields yielding quantities of oospore-infected pods frequently showed almost no downy mildew upon the foliage, attempts were made to obtain the conidial stage of the fungus. Affected pods were placed in moist chambers held at 15° C. and at room temperature, but no fungus outgrowth resulted. Limited attempts under similar conditions to germinate oospores upon healthy, freshly picked pods, and to infect living pods on potted plants in the greenhouse by introducing oospores and mycelium into the ovary cavity were also unsuccessful.

In late October, 1932, a number of pea pods heavily infected with oospores were chopped up and incorporated in the upper part of the soil in half a dozen 6-inch pots. Pea seed of the variety Hundredfold were planted and the pots, along with controls, placed outdoors. No sign of disease developed upon the resulting plants, which were killed by frost in December. In mid-January, 1933, the same pots were replanted in the greenhouse with seed of the variety Laxton's Progress. When the plants came into bloom near the first of March, no disease was apparent, and two of the pots were placed in the saturated atmosphere of a large glass humidity chamber, with several control pots. On examination 6 days later the conidial stage of a Peronospora was found on the stems in a zone extending 2 inches above the soil. None of the plants in the control pots showed any sporulation. One of the pots of infested soil contained 9 plants, 7 of which bore stem infection of downy mildew, and in the other pot, 5 of 6 plants were similarly diseased. A week later the conidial fructification extended to a height of 4 to 5 inches above the ground line, and one small plant, apparently from a seed that had germinated more slowly than the rest, had died, as a result of a systemic infection. In contrast with the sparse sporulation on the plants with infected stems, the systemically infected seedling was densely covered with the velvety, lavender gray mildew. This indicates, although it does not prove, that systemic infection of peas with downy mildew may occur where seed is planted in oospore-infested soil. No other infection developed either in the greenhouse or outdoors, although peas had been grown in the greenhouse during this period continuously. There was no reason to suspect that the infection could have come from any source other than the diseased pods placed in the soil.

Conidia obtained from the stem sporulation on the plants that had become infected from oospores in the soil, were used to inoculate other plants of the same variety. Four pots averaging 7 pea plants each, all in the young-pod stage, were atomized with a suspension of conidia, kept in a humidity chamber 2 days, and then placed outdoors with several pots of noninoculated plants as controls. Within 2 weeks definite symptoms were apparent upon the inoculated plants in the nature of a slight yellowing and dwarfing of the foliage and yellowish blotches on the pods, the latter typical of the blotches on the oospore-infected pods such as had been used in the original soil inoculation. Two of the 4 pots of peas showing symptoms of infection, but devoid of any external growth of the fungus, were stored for about 18 hours in the saturated atmosphere of a humidity chamber, along with 2 pots of the control plants. At the end of this time the young foliage, stems and petioles of several inoculated plants were covered with the characteristic lavender gray masses of conidiophores and spores of Peronospora, whereas no evidence of infection was found on the controls.

Furthermore, a few of the young green pods showed yellowish blotches and bore conidiophores and conidia, although the sporulation was more sparse than upon the foliage. The interior of these pods was found to be coated with oospores and mycelium, as previously described for field specimens (Fig. 1, A). However, epithelial proliferation had not occurred to any noticeable extent, a situation also frequently found in field infections when the fungus entered early in the development of the pod.

The 2 pots of peas that showed symptoms of infection, but had not been placed in the humidity chamber with those just described, had meanwhile been subjected to several cloudy, somewhat humid days outdoors, and upon the smallest, youngest, yellowed leaflets a light, whitish coating had appeared upon the surface. Microscopic examination of the whitish coating proved it to be similar to the mycelial and oosporic coating that had been found on the interior of the infected pods. Apparently the humidity, or some other factor, was sufficiently favorable to encourage the development of mycelium and oospores upon the leaf surface, but not enough to bring about conidial sporulation. Upon placing these 2 pots in the moist chamber along with adequate controls, abundant conidial sporulation occurred.

Beginning, then, with oospores from infected pods it was possible to infect pea plants through the inoculation of soil and to recover the conidial stage with which in turn both the conidial and oospore stages were produced upon the leaves, stems, and pods of still other plants.

Microscopic examination showed that the fungus agreed within reasonable limits with de Bary's (1, p. 112–113) description of *Peronospora viciae,* and also with the measurements given by Gäumann (3) for both *P. pisi* and *P. viciae.* However, since Gäumann reports the oospores of *P. pisi* unknown, and the oospores here described in pea pods seem to compare favorably with those of *P. viciae,* it seems desirable at present to refer to this fungus by the latter name.

FIELD OBSERVATIONS

Market peas grown in Alameda, Monterey, and San Luis Obispo Counties, California, are subjected to a cool humid climate. Even though the days may be warm, the nights are cool, and the plantings are exposed to frequent ocean fogs. Peas are grown extensively in these regions and the growing seasons are long, a condition favorable to the establishment and perpetuation of disease-producing organisms. In these regions downy mildew is common on the foliage during the spring, and often severe. Systemically infected plants occurring at this season suggest either seed infection or seedling infection from infested soil, the conidial masses usually breaking out over the entire plant before the blossoming period. From such infection centers secondary infections of the foliage spread through

the remainder of the field. Abundant inoculum is undoubtedly provided to the soil by infected crops in the form of oospores in plant débris. The fall crop of peas, which may be planted after July, is grown under irrigation and is subject to rains only late in the fall, whereas, with the spring crop, rains occur early and decrease with the approach of blossoming. In the 1932–33 seasons foliage infection during the early fall crop was much less abundant than in the spring crop, yet pod infection was much more troublesome in the early fall crop.

Although oospore-infected pods have been found both in the spring crops and fall crops, there appeared to be a greater amount of pod infection in the fall and the conspicuous symptom of epithelial proliferation was prominent only during the late summer and early fall. Why proliferation should have been so much more abundant on pod lesions in the early fall crop than in the spring crop is perhaps tied up with the environmental complex of temperature, humidity and light, as suggested by Curzi and Barbaini (2) for intumescences occurring on the exterior surfaces of pea pods. It is during this period that plants are subjected to alternate periods of high summer temperatures and cool drenching fogs. It seems that under these conditions of growth the oosporic lesion provided the stimulus for the proliferation of the cells of the membrane on the interior of the pod.

In 1933 the felty pod condition was first observed in a field in Monterey County on peas being picked in mid-August. Casual observation indicated that the field was free from downy mildew, but careful search revealed very small, scattered lesions on the leaves and stipules, bearing only sparse and occasional sporulation. The diseased pods showed the typical yellowish, rather inconspicuous surface blotches. Examination of a number of these showed that within each pod there was a felty layer, ranging up to an inch in extent, and occurring directly under the external blotch. No fungous growth was observed either on the outer surface or in connection with the proliferation, but, on peeling back the felt-bearing membrane, a mass of oospores was found imbedded in the tissue of the pod wall. These lesions were relatively recent, as indicated by their size, and their occurrence was preceded by an extended period of very foggy weather.

It appears from the experiments and observations discussed, that the oospores found in pea pods showing the symptoms described, are those of downy mildew, *Peronospora viciae*. Also it seems that under certain conditions, the presence of the mycelium and oospores of *P. viciae* in the ovary wall may be accompanied by internal proliferations of the inner epithelial membrane, the conspicuous symptoms of which are here described. Although the identity of the oospores has been established experimentally, the suggested relation of epithelial proliferation to the presence of *P. viciae* in the ovary wall remains based upon circumstantial evidence. Repeated

observations of the character already referred to emphasize the probability of the relationship, but experimental methods, taking into account the peculiar environmental factors, will be necessary to prove that *P. viciae* is the cause of this type of cell stimulation. It should be recalled, also, that felty pod is known to occur in response to inciting agencies other than this fungus, both *Cladosporium pisicola* Snyder, and wounds already having been demonstrated as capable of bringing about the phenomenon.

SUMMARY

During certain periods, pods of market peas, *Pisum sativum*, grown in coastal areas of California, sometimes show high percentages of oosporic lesions, unattended by appreciable amounts of foliage infection of downy mildew.

Externally, the infection produces inconspicuous, yellowish blotches. The fungus may remain confined within the pod-wall tissues, in which case it is frequently attended, internally, by a felty proliferation of the epithelial lining, beneath which the oospores lie imbedded, or, the fungus may enter the pod cavity and spread over the inner surface as a mealy layer of mycelium and oospores, in which case the internal intumescences may or may not accompany the fungus.

Incubation of the diseased pods under various conditions has failed to bring about conidial sporulation, and likewise, attempts to infect living pods by direct inoculation with oospores and mycelium have failed.

The conidial stage of *Peronospora viciae* has been obtained, however, by growing plants in soil artificially infested with oospores from diseased pods. With conidia thus produced, abundant infection of healthy plants was obtained yielding both the common downy mildew sporulation on the foliage, the yellowish blotches on the pods, and oospores in the tissues of both diseased foliage and pods.

Incubation of these artificially infected pods in a humidity chamber has yielded a sparse development of conidial sporulation upon the pod surface. Internally such pods have shown a thin white or tan coating of mycelium and oospores lining the pod cavity.

An instance of systemic infection in a seedling grown in oospore-infested soil suggests that a part at least of the systemic infections observed in the field may arise as a result of soil infestation. Also, the presence of mycelium in the seed coats of seed from oospore-infected pods makes possible dissemination of the fungus with seed.

Under certain conditions a superficial layer of mycelium and oospores was produced as a white coating on the youngest leaves, instead of the conidia ordinarily observed under more humid conditions, and resembled the peculiar adaptation to surface growth of the mycelium and oospores

frequently found in the pod cavity spreading superficially over the inner membrane.

Field observations have shown a close association, during certain periods, between white, mold-like, internal intumescences extending into the pod cavity and oospore-containing lesions of the ovary wall, the only external symptom being a yellowish blotch on the pod surface. It is suggested that under certain conditions the infection in the pod wall provides the stimulus for the inward proliferation of cells constituting the internal epithelial membrane. Other agencies are known to incite a similar proliferation.

The observation that pod infection and associated internal intumescences appear most abundantly following heavy coastal fogs presupposes that this environmental factor provides the opportunity for infection and also favors the development of the intumescences when the necessary stimulus is present.

UNIVERSITY OF CALIFORNIA,
BERKELEY, CALIFORNIA.

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THE CONFINEMENT OF CHLOROPICRIN AND OTHER GASES FOR FUMIGATION PURPOSES[1]

G. H. GODFREY

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In connection with studies on soil fumigation for nematode control reported elsewhere (2, 3, 4), it was found that chloropicrin was efficient in proportion to the efficiency with which it was confined in the soil. Experiments in which the opportunity was available for it to evaporate quickly gave very poor results in nematode control, whereas cases in which the gas was confined by means of a fairly gas-tight cover gave excellent control. These observations led to studies on the penetrability of the gas through covering materials of various kinds. Details and results of these studies are herein reported.

METHODS

The method used for the study of permeability of membranes to chloropicrin gas was a purely empirical one, consisting of the insertion of the chemical in liquid form into ordinary 4-inch Petri dishes covered with the membrane sealed tightly in place at the edges, and then determining the rate of evaporation by reduction in weight. Any kind of paper, even cheap newsprint paper, proved satisfactory as a base upon which to apply coatings of the different materials to create the experimental membranes. Eventually, however, 40-pound Kraft paper was used almost exclusively because of its strength and freedom from troublesome curling.

Detailed procedure was as follows: The paper for the cover, cut in circles of about $\frac{1}{2}$ inch greater diameter than the dishes, was glued tightly in place, well over the edges. The cover was then painted with the coating material in liquid form and the dishes were set aside for the coatings to dry. The attainment of constant weight was considered as sufficient drying. Variations of a few milligrams thereafter, with changes in air humidity, were not sufficient to alter major conclusions. Chloropicrin was inserted by means of a burette with capillary outlet through a pinhole made in the cover. As a rule, exactly 5 grams were so inserted. Slight adjustments to bring about exactness were made with the dish on the balance by means of a small capillary medicine dropper. Immediately thereafter the pinhole was covered with a small piece of adhesive paper that was then coated with the same material used on the cover. The dishes were laid aside and weighed at frequent intervals and losses in weight due to evap-

[1] Published with the approval of the Director as Technical Paper No. 59 of the Experiment Station of the Association of Hawaiian Pineapple Canners, University of Hawaii.

oration of the chemical were recorded in decigrams. With the initial weight of the chemical exactly 5 grams these figures were converted readily to percentage loss by multiplying by 2.

The temperatures of exposure throughout the tests were fairly uniform, ranging between 22° and 28° C. with an average of about 25°. It is recognized that the vapor pressure of chloropicrin, as of other volatile compounds, varies greatly with the temperature and that more reliable results would have been attained had a constant temperature been maintained. Lacking equipment for this, the writer had to be content with his records that showed satisfactorily the wide differences in permeability of different coatings, and permitted him to select those of highest efficiency for confining the gas. At 25° C. the vapor pressure of chloropicrin is 23.97 mm. (1). This pressure, except for the greater or smaller variations due to temperature changes (maximum range, 20 to 28 mm.), may be considered to have remained relatively constant in the small chamber used (about 80 cubic centimeters capacity) until the liquid inserted had completely evaporated. Any partly pervious membrane used as a cover should theoretically permit a fairly uniform rate of penetration of the gas. With a very permeable membrane the rate of loss may be expected to be rapid; with a highly impervious one the rate is slow. Paper covers alone permitted of very rapid evaporation, the material disappearing completely as a rule in about 2 hours.

MATERIALS USED AND RESULTS

As with the method, so with the choice of materials, procedure was at first purely empirical. Various materials were tested as paper coatings, with widely varying results. Paints and varnishes of various kinds were found to be relatively inefficient. Losses of 90 per cent or more occurred in less than 24 hours with coatings of oil house paints, Valspar varnish, amyl lacquer, a commercial black enamel, a graphite roof paint, "Convole" rubber, and various kinds of rubber latex. Ordinary building cement and a waterproof building cement showed total loss in 2 to 4 hours. Shellac showed 88 per cent loss in 24 hours. It was arbitrarily considered that if 50 per cent loss occurred in 48 hours or less the material used might well be eliminated from further consideration. This, of course, threw out all those just mentioned.

Cellophane, used in place of paper, was found to be highly efficient for confining chloropicrin. Aside from its uncertain durability for the purpose proposed this material is far too expensive for practical use. Its efficiency, however, led to tests with cellulose acetate preparations. Coatings of Celanese on paper in a very thin layer showed only 26 per cent loss (13 decigrams of the original 50) in 48 hours, 40 per cent in 72 hours, and

64 per cent in 6 days. A slightly heavier application showed somewhat greater efficiency. While fairly effective for the purpose in mind, this material, also, is too expensive for general use.

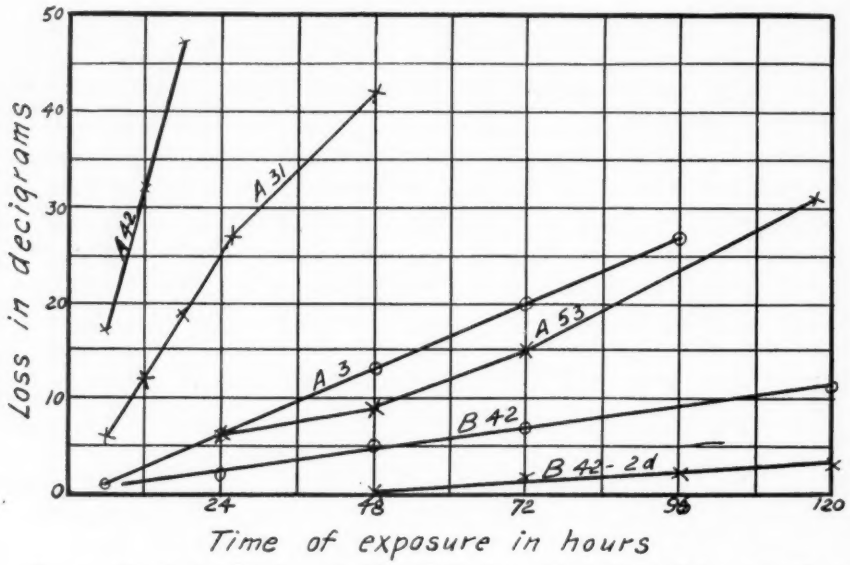

FIG. 1. Graph showing loss of chloropicrin from 4-inch Petri dishes covered and sealed with several different membranes. A42, a representative tar-impregnated mulching paper. A31, paper coated with shellac. A3, Celanese, very thin application; note uniform rate of loss. A53, water glass; note more rapid loss after 48 hours, due to development of very fine crackings. When dishes were placed out of doors, subject to greater fluctuations of temperature and moisture, cracks developed quickly and total loss occurred in a few hours. B42, Golden Ground hoof and horn glue. B42—2d, same dish repeated 6 weeks later, showing apparent improvement with age. The carbon bisulphide line with membrane B126, Casein glue, would fall just above that for B42, with 18 decigrams loss in 120 hours. All other covers tested showed losses of over 50 per cent in less than 64 hours (all but one in less than 36).

Sodium silicate (water glass) proved to be very highly effective as a paper sizing material in laboratory tests, as described. Its efficiency, in comparison with other materials, is graphically shown in figure 1. Its cost, moreover, is very low. Water glass, however, has the serious disadvantage of cracking badly when exposed to temperatures and humidities of high variability. This cracking leads to immediate inefficiency for holding chloropicrin. Unless this can be overcome, water glass-coated papers are completely impracticable for confining fumigants more than a few hours.

Of all the materials tested, certain adhesive hydrophyllic colloidal substances, including glues of various kinds, starch paste, and gelatine, proved very highly efficient for holding chloropicrin. Actual results obtained

with some of the better applications are shown in table 1. Some of the results with less efficient materials also are given by way of comparison. Data on tests of the penetrability of the usual pineapple-field, tar-impregnated mulching papers likewise are shown.

It is well to note, in interpreting this table, that the original quantity

TABLE 1.—*Rate of penetration of chloropicrin gas through various covers. Standard conditions, 5 grams chloropicrin in 4-inch Petri dish, covers glued tightly in place; dishes held at room temperature, averaging 25° C.*

Test No.	Covering material	Loss of Chloropicrin, in decigrams							
		Hours							
		6	12	18	24	48	72	96	120
A38	40-lb. Kraft paper	Completely evaporated in less than 2 hours							
A39	Pabco #104 Mulch paper	10	23	36	50				
A40	" #107 " "	8	16	24	28	46			
A42	" #T140 " "	17	32	47	50				
A29	Greenhouse paint	17	35	50					
A31	Shellac, thin	6	12	19	26	42			
A33	"Convolc" rubber	50							
A36	Valspar varnish	50							
A19	Paraffin	9			16	26			
A3	Celanese	1			6	13	20	27	
A52	Water glass	2			6	13	22		39
A53	"	1			6	9	15		31
A55	"	1.5			8	12	16		26
B6	Water glass	1			4		11		
B7	" (exposed to sun)		47						
B8	" " "		47.5						
B42	Golden Ground glue	0			2	5	7		11
"	Same dish, 6 wks. later	0			0	0	2	2	3
B44	Golden Ground glue	1			3.5	9.5	11	14	17
"	Same dish, 6 wks. later				0	1	4	5	9
B53	Glue + Ebonol				0	2.5	4.5	5	
B56	" "				1	3	6	15	
B67	Glue + water glass, 10 per cent				4	6	16		16
B79	Casein glue				0	0	2	3	4
B80	"				0	0	3	6	9
B105	Peerless Ground glue + formaldehyde				0	0	3	7	8
B142	U. S. Govt. Waterproof glue				3	12	13	15	21
B147	" "				0	6	9	14	18
B151	Starch paste				1	7	10		20
B154	Gelatine				0	4	11	15	18

of chloropicrin inserted was always 5 grams. The readings of losses, in decigrams, therefore, give an immediate picture of the proportion of the chemical that has disappeared at any particular time. Multiplying the figures by 2 gives this proportion in terms of percentages. Scanning the 48-hour column, for example, shows the covers that were poor and those that were efficient in holding the chemical up to that period. The figures in the final column show the very high efficiency of some of the materials used, with less than 10 per cent loss indicated in 5 days of exposure.

The table shows only a few representatives of each of the various lots of material tested. The mulching papers used in pineapple-plantation practice, while certainly holding the gas to some extent, were not highly efficient for the purpose, with idicated losses of 100 per cent or nearly so in 48 hours or less. The second group in the table shows the very low order of efficiency of paints, varnishes, rubber coatings, and paraffin. Various other materials of similar natures were tested with like results. The third group includes materials of apparently high efficiency but objectionable from other points of view, such as cost or durability. The final group, B42 to B154, includes the range of materials tested that show, as a rule, high efficiency for retaining chloropicrin, and that are otherwise practicable.

In an effort to develop a coating that would make the paper more or less weatherproof as well as gas-impervious, combinations of various materials were tested. Some of the U. S. Forest Products Laboratory waterproof glues (5) proved to be highly retentive of the gas. Ordinary glues sprayed or brushed with formaldehyde solution were rendered waterproof to some extent, without material change in their permeability. Supplementary coatings of waterproofing materials, such as the tar paint, "Ebonol," did not alter materially the chloropicrin-holding capacity. In test No. B53, in which a light grade of Kraft paper (Graham Paper Co. 15 lb. Kraft) was first treated with Golden Ground animal-product glue, thoroughly dried, and then coated with Ebonol, the loss was virtually nil in 96 hours. Forest products casein glue, formula No. 11 (5) was fairly efficient and, at the same time, somewhat waterproof. Le Page's casein glue, applied as a thin coating, was one of the best treatments used. There was some tendency, however, for the casein glues to peel away from the paper upon exposure, whereas such tendency was entirely lacking in the ordinary hoof-and-horn glues, which, however, are not of themselves so waterproof.

The ordinary hoof-and-horn glues were from many points of view the most practicable of the materials tested. Many different kinds and grades were approximately the same in chloropicrin-holding capacity. An apparent increase in efficiency with age is evident in the comparison of the two

coatings, numbers B42 and B44, with the same two tested 6 weeks later. The lower grades are highly efficient, easy to apply, and inexpensive.

Figure 1 shows graphically the results obtained with representatives of the different materials tested. In general, in accordance with theoretical considerations, the rate of diffusion of chloropicrin through the various unbroken membranes was fairly uniform, and, consequently, the plotted lines are approximately straight. Slight variations are explainable by variations in moisture absorption of the covering material and, of course, by temperature differences. In certain cases, however, deviations from the straight line are quite marked. For example, the water-glass-covered dishes, as a rule, showed more rapid diffusion with the passage of time, and this is indicated in figure 1 by the greater steepness of slope of the lines after the second or third day of exposure. This is due no doubt to the before-mentioned cracking of the water-glass membranes. Exposure of dishes to the sun for an hour or two hastened the cracking and the consequent escape of the gas.

Several of the coated papers were tested for permeability to certain other gases. It was found that carbon bisulphide penetrated most membranes much more readily than did chloropicrin. Figure 1 shows results from a single test. Water glass, rubber, ordinary glue, in fact, most of the materials tested were very inefficient. The Forest Products Laboratory casein glue, interestingly, was the only material tested that was highly efficient. Several repetitions with this glue were equally good. In view of the rather high vapor pressure of this gas (361 mm. at 25° C.) this finding is encouraging as to possibilities. Tetrachlorethane was very much like chloropicrin in its ability to penetrate membranes. Glue coatings of various kinds were efficient to the extent of permitting only very small losses, ranging from 8 to 18 per cent, in the course of 5 days. Hydrocyanic acid gas diffused rather rapidly through practically all the membranes that were tested. No quantitative tests were made with this gas, judgment as to rate of diffusion being based solely on the rate of coloration of Guayacum-copper sulphate test papers. Obvious differences existed, and, inasmuch as this gas is used so extensively in fumigation, further studies would appear to be justified.

APPLICATION

In experiments on soil fumigation for nematode control, reported elsewhere (3), the writer has obtained better than 99 per cent control with chloropicrin in a large number of cases in which the soil was confined in closed containers sealed with special covers of gas-impervious papers. Perfect control was obtained in several cases. In nematode-control experiments in pineapple fields in Hawaii (2), strikingly better results were obtained with the use of mulching paper covers over treated soils than when

no covers were used (observations without quantitative data). What might be obtained with a highly impervious cover in place of the less efficient mulching paper (see present results) remains to be determined. For field applications the chemical is first applied in the soil, immediately after which the unbroken paper is applied over the bed with the edges buried as deeply as practicable (6 or more inches if possible) and the soil well firmed over the edges. Wetting the soil at the edge of the paper, where practicable, is another precaution contributory to better results in that it helps to prevent the outward diffusion of gas away from the place it is needed. A tight paper cover definitely makes a difference of several days in gas retention in the soil. For special applications, such as seed-bed or greenhouse-bed fumigation or treatment of isolated spots in a field, the use of a metal or wood frame sunk into the ground around the plot to be treated, with the paper cover tightly sealed to the frame all the way round, is indicated. In one experiment in which this was done with only moderate efficiency (3), excellent results in nematode reduction and improved growth of tomato plants were obtained.

The same gas-impervious paper has been used to advantage for insect control. The writer constructed of wood a light box frame 2 by 2 by 2 feet in dimensions, and covered it with Kraft paper, coated inside and out with glue. A similarly covered lid was so built as to fit tightly into place with the line of junction flush with the surface of the frame. In a practical test with this miniature "fumigation chamber" a small piece of bamboo furniture infested with a small wood-boring beetle (species undetermined) was placed in the box; an open dish containing about 5 cubic centimeters of chloropicrin was inserted and the cover sealed in place with strips of adhesive manila tape. After sealing no odor of chloropicrin was apparent at any time about the box. The lid was removed after one day and a very strong odor of the gas was still apparent in the box. Complete efficiency in killing the insects was attained. Similar fumigation chambers up to 1000 cubic feet capacity have been constructed by the writer, and have been used to practical advantage for the killing of stored-product insects of various kinds.

The technique of applying the gas-impervious coatings is simple, and the costs of the cheaper materials surprisingly low. The writer has applied glue sizings by either brushing or spraying the glue in 12 or 15 per cent concentration (by weight) in warm water. For large-scale applications he has successfully used a dipping process, unrolling 4-foot Kraft paper directly through a specially constructed vat containing the hot liquid glue (10 per cent concentration is satisfactory for this) and lifting it, to dry, with an overhead pulley and rope arrangement. In this way he has frequently treated strips 30 feet long in only a minute or two. One pound

only of a glue retailing at 15¢ per pound was sufficient to coat adequately, on both sides, 500 square feet of paper.

SUMMARY

Concurrent with soil-fumigation tests for root-knot-nematode (*Heterodera marioni*) control reported elsewhere, studies were made in the nematology laboratory of the Experiment Station of the Association of Hawaiian Pineapple Canners, at Honolulu, of means of confining fumigation gases to increase their killing efficiency. Tests were conducted by inserting measured quantities of the volatile chemicals used, in liquid form, into 4-inch Petri dishes previously sealed with covers of paper coated with the various membranes to be tested, and recording losses in weight with the passage of time. Paper alone was wholly inefficient in confining the gases. Papers covered with thin continuous films of various adhesive hydrophyllic colloids, particularly glues of various kinds, gelatine, and starch paste, were highly efficient in confining chloropicrin. Cellulose acetate was likewise good. Water glass was highly efficient in the laboratory at relatively uniform temperatures but cracked quickly when exposed to the sun, thereby immediately losing its efficiency. Ordinary animal glue actually improved with age. United States Forest Products Laboratory casein glue was the only material that was highly efficient in confining carbon bisulphide. Tar impregnated commercial mulching papers were very poor in efficiency. Papers covered with oil-containing paints and varnishes were very poor, most of the gas diffusing through them in much less than 24 hours. Animal glue being cheap, durable, and highly efficient for confining chloropicrin, may be used as a sizing on paper to produce a relatively gas-tight covering material applicable for general fumigation purposes. Practical applications with excellent results have been made experimentally in soil fumigation for nematode control, and in grain fumigation for the control of weevils and moths of various kinds.

UNIVERSITY OF CALIFORNIA,
BERKELEY, CALIFORNIA.

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TRICHODERMA SHEATH SPOT OF RICE

E. C. TULLIS[1]

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INTRODUCTION

The disease referred to herein as the "Trichoderma sheath spot" of rice was first called to the writer's attention during the latter part of the summer of 1929 by J. Mitchell Jenkins, of the Rice Experiment Station, Crowley, La. A few miles north of Crowley a field of Blue Rose rice was inspected in which the disease was so severe that all the lower leaves had been killed as a result of the lesions on the sheaths. Subsequently, the disease has been found in different degrees of severity on various commercial varieties of rice in Louisiana and east of the Brazos River in Texas. In the fall of 1932 it was found for the first time in Arkansas, just west of Roe, on Early Prolific rice growing on "new" land.

SYMPTOMS

The lesions appear first on the sheaths at about the water line, in mid-July. When the lesions first become visible they are reddish brown, 2 to 3 mm. long by about 1 mm. wide. Within a relatively short time, however, they enlarge considerably and their centers become lighter in color ("cream," according to Ridgway[2]) and the margins remain reddish brown ("light seal brown," according to Ridgway). Various stages in the development of the lesions are shown in figure 1.

The central portion of the spot is irregular in outline, owing to the fact that the tissues over the vascular strands do not bleach out so readily as those between the strands. This is shown in the specimen at the extreme right in figure 1. Frequently, when abundant secondary infections appear, the central portion of the spots do not bleach out so readily as in earlier infections, and the whole spot may then vary from a grayish brown at the center to the darker seal brown of the border. As may be seen in the specimen at the extreme right in figure 1, the darkest portion of the border is immediately adjacent to the bleached-out portion. Old lesions frequently attain a length of 10 cm. and, late in the season, may be found on the sheaths from the water line to the base of the flag leaf. In a few cases the fungus has been observed to produce a definite leaf stripe, extending from the base to the tip of the leaf, apparently a continuation of a sheath infection.

[1] Agent, Division of Cereal Crops and Diseases, Bureau of Plant Industry, U. S. Department of Agriculture. Investigations conducted in cooperation with the Louisiana, Texas, and Arkansas agricultural experiment stations.

[2] Ridgway, Robert. Color standards and color nomenclature. 53 col. pls., 1115 named colors. Washington, D. C. 1912.

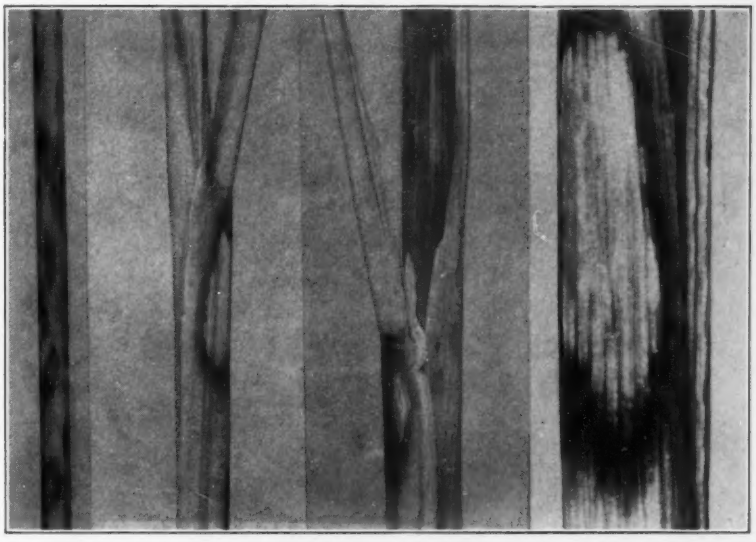

FIG. 1. Various stages in the development of the lesions caused by *Trichoderma lignorum* on rice sheaths. Three specimens at left, ×1; that at right, ×3.

VARIETAL SUSCEPTIBILITY

A marked difference has been observed in the reaction of varieties to infection by this fungus. The most susceptible varieties, listed in the order of their relative susceptibility, are Vintula, Carolina Gold, C. I. 2971, Fortuna, Rexoro, and Blue Rose. Other varieties are attacked at times but not so severely as those listed above. The short-grain varieties, as a group, exhibit considerable resistance to the disease. On plants of Vintula and Carolina Gold it is not unusual, at heading time, for the fungus to have attacked the sheaths and thus killed all but the 2 uppermost leaves. Healthy plants of these varieties normally have from 6 to 8 vigorous functional leaves at this period. Invasion of the culm by this fungus seems rather rare. The greatest damage to the plant is due to the reduction of leaf area at a critical stage in the development of the plant.

CULTURAL CHARACTERISTICS OF THE FUNGUS

Tissue platings of the lesions in the sheaths have been made each growing season since 1929, and in nearly all cases the same fungus was isolated. From the diseased tissue there is produced a white, rapidly growing mycelium. On corn-meal agar, the colonies, either from tissue platings or from transfers, usually cover the surface of the agar in 2 or 3 days. As soon as spore production begins, the aerial portion of the colony changes rapidly

to a color comparable to Ridgway's French green.[3] On bean-agar or beef-extract peptone agar the colonies remain white. However, sporulation does not occur on these media.

IDENTIFICATION AND DESCRIPTION OF THE FUNGUS

The fungus was identified as belonging in the genus Trichoderma[4] and has been tentatively designated as *Trichoderma lignorum* (Tode.) Harz.

Mycelium hyaline, septate, 6 to 10 μ; conidiophores mostly 6 to 8 μ in diameter, dichotomously or trichotomously branched, diminishing in size toward the tip; conidia in heads, spherical to subspherical, borne on short verticillate branches that are much constricted at the apex and 2 to 6 μ in diameter (Fig. 2).

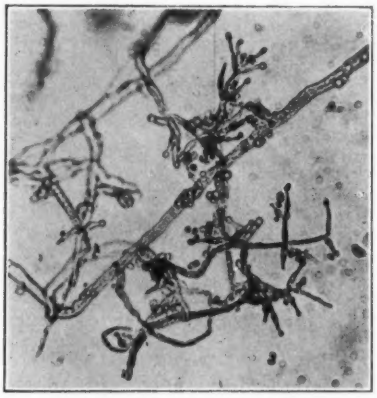

FIG. 2. Mycelium, conidiophores, and spores of *Trichoderma lignorum* from corn-meal agar culture. ×80.

PATHOGENICITY OF THE FUNGUS

Inoculation experiments were conducted in the greenhouse on plants of the Supreme Blue Rose and Carolina Gold varieties.

The plants were grown in Clarksville silt-loam soil, from the experiment station farm near Fayetteville, Ark., in 3-gallon stoneware jars. About 10 plants of each variety were grown in each of 4 jars. The plants of both varieties in each of 3 of the jars were inoculated, when about half-grown, with conidia from pure cultures of the fungus. Approximately 20 per cent of the plants became infected. The lesions on the sheaths were typical of the disease as it occurs in the field and the fungus was reisolated from the

[3] Ridgway, Robert. (See footnote 2.)

[4] Determination of the genus was made by Miss Vera K. Charles, Division of Mycology and Disease Survey, Bureau of Plant Industry, U. S. Department of Agriculture.

infections produced on these plants. No secondary infections were produced on these plants in the greenhouse. The noninoculated plants remained healthy.

PATHOLOGICAL HISTOLOGY

Portions of typical lesions on sheaths of Blue Rose rice were placed in strong chromo-acetic fixative for 12 hours, washed, treated with strong hydrofluoric acid for 6 hours, and washed again to remove all traces of the acid. The specimens were then dehydrated in ethyl alcohol, according to the usual procedure, until 70 per cent of alcohol was reached, and further dehydration was accomplished by means of a modified butyl alcohol-ethyl alcohol series, as described by Zirkle.[5] They were then infiltrated with paraffin and embedded. Sections were cut 20 μ thick and stained with Heidenhain's iron-alum haematoxylon.

An abundance of mycelium was found in the parenchyma tissues in the region of the aerenchyma of the sheaths, and large coarse hyphae of the fungus were found traversing these spaces. The tissues of the invaded areas had been killed by the fungus, as evidenced by their discoloration and the collapse of the cells. The tracheal tissues also were attacked, as shown by the ramification of the mycelium in the vascular elements. There was a greater abundance of mycelium in the tissues on the inner portion of the sheaths.

SUMMARY

A previously undescribed sheath disease of rice has been found chiefly in Louisiana and Texas and to some extent in Arkansas.

The disease has been found on most of the commercial varieties of rice grown in Louisiana and Texas. Some varieties exhibit greater susceptibility than others.

The causal fungus is tentatively designated as *Trichoderma lignorum* (Tode.) Harz.

A limited number of inoculation experiments in the greenhouse have demonstrated the pathogenicity of the fungus.

The fungus invades the parenchyma and vascular system of the sheaths and kills the attacked portions.

[5] Zirkle, C. The use of N-butyl alcohol in dehydrating woody tissue for paraffin embedding. Science, n.s. **71**: 103–104. 1930.

PHYTOPATHOLOGICAL NOTES

The Susceptibility of the Peach to Artificial Inoculations with Bacterium Syringae and Some Allied Organisms.—During the course of some comparative studies of the plant pathogenic bacteria listed in table 1, aqueous suspensions of the organisms were injected into the leaves and succulent shoots of the peach, *Prunus persica,* at various times in 1932, 1933, and 1934.

TABLE 1.—*Organisms used in inoculation experiments to determine the susceptibility of the peach to Bacterium spp.*

Organism	Host	Source	Isolated by
Bacterium syringae	Lilac	Holland	M. K. Bryan
Bacterium syringae	Lilac	U. S. A. (Illinois)	M. K. Bryan
Bacterium prunicola	Cultivated plum	England	H. Wormald
Bacterium morsprunorum	Cultivated plum	England	H. Wormald
Bacterium papulans	Apple (Stayman variety)	U. S. A. (Arkansas)	J. C. Dunegan
Bacterium sp.—Target-canker organism	Apple (Delicious variety)	U. S. A. (Virginia)	J. W. Roberts
Bacterium sp.—Italian prune leaf-spot organism	Italian prune	U. S. A. (Arkansas)	J. C. Dunegan

In making the inoculations on the leaves, a hypodermic needle was drawn slowly along the under surface on both sides of the midrib, making two narrow, torn lines in the lower epidermis through which the bacteria were forced into the mesophyll tissue. The symptoms developing from such inoculations appeared within 3 to 5 days and consisted of a very light green (almost white) bleached streak surrounded by purple margins along the needle path. The leaf tissue in the chlorotic streak eventually broke away from the healthy tissue.

The organisms were injected into the twig tissues by carefully inserting the hypodermic needle under the epidermis and forcing the suspension into the tissues. A circular bleached area with a purple margin generally appeared in the immediate vicinity of the needle puncture within 3 days. When the punctures were close together coalescence of the margins gave the twig a very striking appearance.

Adequate controls were maintained for all the experiments and no such symptoms as those just described developed following the injection of the

leaves and twigs with sterile water. As a further control, parallel inoculations were performed with cultures of *Bacterium pruni* and, while symptoms typical for this organism appeared on the leaves and twigs, no pronounced chlorotic symptoms developed.

The results of the inoculation experiments demonstrate that the peach is susceptible to various bacteria listed in table 1 when the bacteria are actually introduced into the tissues of the leaves and twigs. The symptoms indicate a marked effect on the chloroplasts in the immediate vicinity of the point of inoculation and a stimulation of anthocyanin pigment formation in the surrounding regions. That these symptoms were induced by these particular bacteria is demonstrated by their nonappearance in the control experiments.—JOHN C. DUNEGAN, Division of Fruit and Vegetable Crops and Diseases, Bureau of Plant Industry, U. S. Department of Agriculture, cooperating with the Department of Plant Pathology, University of Arkansas.

Alternaria brassicae as a Parasite of Chinese Cabbage.—For the past decade, Chinese cabbage plants (*Brassica Pe-tsai* Bailey) growing in Massachusetts have been parasitized by an organism similar to an Alternaria. However, some uncertainty existed concerning the parasite, so an investigation was undertaken to determine the Latin binomial, the hosts, and the life history of the fungus. This work was terminated and its publication delayed; lately, however, a request has been made for reporting the results.

The manuscript is accompanied by one plate and two text figures and seems unnecessarily long, so it will be retained for reference, in case future questions arise, and only the summary presented here.

Chinese cabbage, in Massachusetts, was seriously parasitized by an organism producing symptoms associated with an Alternaria or a Macrosporium. A description in literature of Alternaria or Macrosporium parasitizing Chinese cabbage had not come to the writer's attention. Cultures showed the parasite to be an Alternaria. The common name suggested for the disease, therefore, is Alternaria leaf spot of cabbage. The species of the organism could not be definitely determined by spore measurements and other morphological characters, since these varied so widely that no standard measurements were at hand. A need for more accurate measurements of conidia with regard to pedicels and beaks is discussed. The species was principally determined by the results of the inoculations. Inoculations within the species and reciprocal inoculations showed that this organism is physiologically identical with *Alternaria brassicae*, which commonly causes the Alternaria leaf spot of common cabbage.

This Alternaria varied morphologically from descriptions of *A. bras-*

sicae as follows: a. The conidia were generally elliptical or fusiform. b. Longitudinal septations were plentiful in the conidia. c. The conidia were blunt or rounded. d. Measurements of conidia showed wider limits of variation: 5–29 × 7–102 μ, as compared with 10–30 × 35–120 μ for those of *A. brassicae*. e. Some of the conidiophores were wavy. These differences were not considered sufficiently outstanding to establish a separate species or a form as *A. brassicae* f. *Pe-tsai*.

Following is a description of the fungus: *Hyphae:* Long, rather straight, profusely branched when actively growing; sparsely branched in old cultures; cells, 4–6 μ wide by 5–30 μ long; at first hyaline to yellow, turning olivaceous to russet and cinnamon. *Conidiophores:* medium length, sometimes tortuous, russet or cinnamon brown; branched or unbranched; each branch arising near the host tissue or substratum, of nearly uniform length, averaging 5 cells, 4 × 55 μ; when supporting single large conidia, 3.5–7 × 80–145 μ. *Conidia:* cinnamon brown, no echinulations or rugae; apical portion tapering, rounded to blunt; both catenulate and single; shape, obclavate when catenulate; muriate; longitudinal septations common in those with 5–6 cross walls; limits of variation from dried Chinese cabbage leaves, 5–29 × 7–102 μ; sizes of conidia containing different number of cross walls are reported in table 1, lot 3, and need not be repeated here. *Hosts:* Chinese cabbage and common cabbage, cauliflower. *Noninfectants:* Horseradish, turnip, collards, mustard, brussel sprouts were not infected when inoculated. The parasite is *Alternaria brassicae* (Berk.) Sacc.—W. H. DAVIS, Department of Botany, Massachusetts State College.

Peach Mosaic.—Specimens of peach branches were received from the Grand Junction area of Colorado in July, 1932, from Mr. E. W. Bodine of the Colorado Agricultural College, for comparison with the red-suture disease, found in Michigan. These specimens showed little, if any, resemblance to branches of trees affected with red suture. Buds from the Colorado material were grafted into a young Hale peach tree, No. 37–2, in the botany plots at East Lansing. The bud wood was in poor condition and a union appeared doubtful. At the close of the season the grafted buds were dead.

In the spring of 1933, tree No. 37–2 showed a crinkling of the leaves and a mosaic type of mottling on all of the early formed leaves. These symptoms approximated those described by Hutchins[1] for peach mosaic. As the season of 1933 advanced, the symptoms became rather obscure with the new growth appearing normal. However, those leaves that developed during periods of low temperatures, such as occurred at East Lansing between June 12 to 18, 1933, showed marked crinkling and mottling.

[1] Hutchins, Lee M. Peach Mosaic. Abst. Phytopathology 23 (1): 17. 1933.

A number of seedling trees were grafted in August, 1933, with buds taken from tree No. 37–2. Three of these trees were transferred to tubs and removed to the greenhouse in December, 1933. The greenhouse temperatures were high, usually above 75° F. No peach-mosaic symptoms were apparent at these temperatures. Symptoms were finally produced on two trees, one of which was placed close to an outside door in a cooler location and the other in a cool house with approximate temperatures ranging between 40° and 50° F.

In May, 1934, the three trees in tubs were removed outside the greenhouse. Again, those leaves formed during the cool periods showed mosaic and crinkling symptoms, whereas the growth produced in warmer weather appeared normal. All of the other bud-inoculated trees remaining in the field developed characteristic symptoms in 1934. It is thus apparent that peach mosaic has a low temperature optimum for symptom expression. Perhaps this explains Hutchins' statement[2] that the mosaic character of the leaves may be fugitive.—DONALD CATION, Michigan State College, Agricultural Experiment Station, East Lansing, Michigan. Contribution No. 69, Michigan Agricultural Experiment Station.

Dissemination of Angular Leaf Spot of Tobacco[1] *by the Southern Tobacco Worm.*—Rain, accompanied by wind, is probably the usual means of spreading angular leaf spot of tobacco (*Bacterium angulatum* Fromme and Murray) from leaf to leaf and from plant to plant in the field. It has been suggested that insects may be possible carriers of the disease, but positive evidence is wanting. In 1934 several leaves of White Burley tobacco were found with angular-leaf-spot infections in two parallel lines (Fig. 1, A) rather than scattered at random, as is usual (Fig. 1, B). Leaves were then found that were partially eaten by larvae of the Southern tobacco moth, *Phlegethontius sexta,* on which it was possible to trace the angular-leaf-spot infections from the borders of the eaten areas for some distance across the uneaten portions of the leaves (Fig. 1, C and D).

The correspondence in distance between the almost straight parallel lines of angular leaf spot and the width between the abdominal legs of the tobacco worm, and the fact that the abdominal legs are used for clinging to and moving over the surface of the leaf indicate that in this case the bacteria were spread mainly from the legs of the worm. The abdominal legs, 5 sets of 2 each, are equipped with semicircles of hooks that cause slight, though not visible, injury to the leaf. The wounds make excellent infection courts for bacteria, especially if moisture be present. The thoracic legs are

[2] See footnote 1.

[1] The investigation here reported relates to a project of the Kentucky Agricultural Experiment Station and is published by permission of the Director.

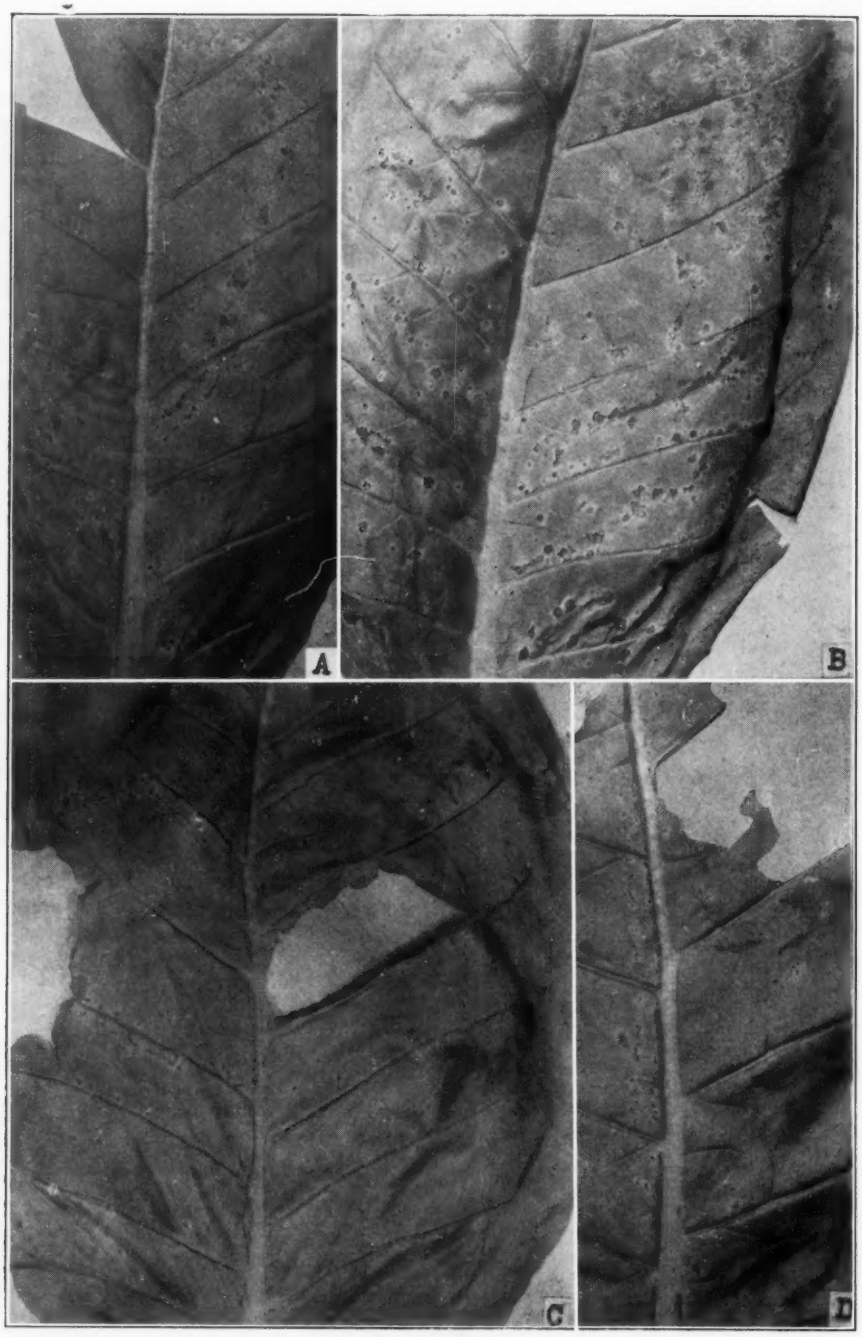

FIG. 1. Angular leaf spot of White Burley tobacco. A. Parallel lines of angular leaf spot on an uneaten leaf. B. Usual random distribution of angular leaf spot. C. and D. Parallel lines of angular leaf spot on leaves of tobacco partially eaten by the tobacco worm.

moved as feelers to the right and left of a straight line and do not seem to be concerned in spreading angular-leaf-spot bacteria.

This method of dissemination of angular leaf spot probably does not occur except when the leaves are damp with dew or rain, so that bacteria can move out of infected areas.—E. M. JOHNSON, Kentucky Agricultural Experiment Station.

Copper Analysis of Foliage Sprayed with Cuprous Oxide.[1]—The ideal for a protective fungicide specifies insolubility, because the chemical must remain on the plant for protracted time periods. The insolubility of cuprous (red copper) oxide suggested its use as a foliage protectant.[2] While investigating that problem, this factor of insolubility was rather forcibly demonstrated when an attempt was made to determine quantitatively the copper on sprayed foliage. The cuprous oxide would not come off in the 0.2 per cent nitric acid solution commonly used for removing copper from leaves, although this solution works beautifully with Bordeaux mixture. Obviously, the cuprous oxide is more insoluble in dilute acid than is Bordeaux mixture. To remove the cuprous oxide, overnight immersion is necessary and this frequently results in the extraction of coloring matter from the leaves, which seriously interferes with the subsequent colorimetric analysis. It also requires too much time and glassware.

The addition of 1 per cent of hydrogen peroxide (30 per cent solution) to the 0.2 per cent nitric acid solves the difficulty. Probably the cuprous oxide is oxidized to the cupric form, which is more easily soluble, and a 30-minute immersion is generally sufficient to remove all the cuprous oxide. The usual procedure for the colorimetric analysis of copper in dilute solution can then be followed.—L. L. ISENHOUR and JAMES G. HORSFALL, New York State Agricultural Experiment Station, Geneva, N. Y.

Physiological Spotting of Pea Seed.—A spotting of pea (*Pisum sativum* L.) seeds, apparently nonparasitic in origin, has been observed since 1930 on peas grown in Wisconsin, Idaho, Montana, California, Sinaloa, Mexico, and under greenhouse conditions at Rosslyn, Virginia. This spotting has been observed only in the Surprise variety and in closely related types such as Peerless (Improved Surprise), Wisconsin Early Sweet, Early Canner, and in crosses between Surprise types and other varieties.

The spots occur only on the seeds, the pods being normal in every respect. When the seeds have about reached the canning stage, the spots ap-

[1] Approved by the Director of the New York State Agricultural Experiment Station for publication as Journal Paper No. 42, June 14, 1934.

[2] Horsfall, James G. Red oxide of copper as a dust fungicide for combating damping-off by seed treatment. New York (Geneva) Agr. Exp. Sta. Bul. 615. 1932.

pear as small somewhat circular water-soaked areas that later enlarge slightly in size. When the seeds become dry the discolored areas are very conspicuous (Fig. 1), in that they are darker green and in some cases al-

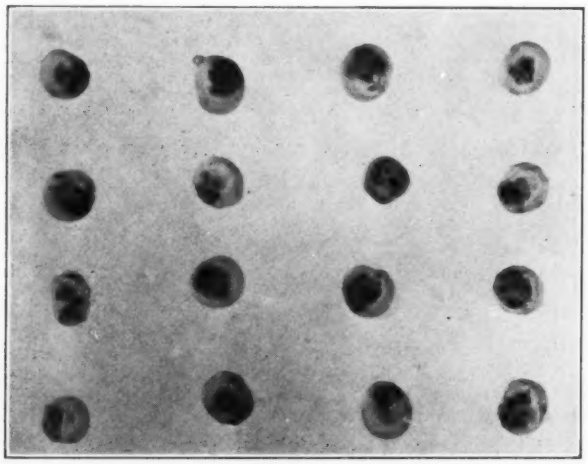

FIG. 1. Lesions showing physiological spotting on seeds of the Wisconsin Early Sweet variety of peas.

most black as compared with the normal green. In general the spotting resembles the lesions caused by the bacterial-blight organism of peas, *Bacterium pisi* Sack., but attempted isolations from such seed have yielded negative results. Spotted seeds are of normal size and germinate just as well as healthy ones. Not all the pods on a plant produce spotted seeds; neither are all the seeds in a pod necessarily affected. In fact, in some cases only one seed in a pod may show the spotting, while in other instances all the seeds may be affected.

Normal seed may, under favorable environmental conditions, produce spotted progeny. On the other hand spotted seeds do not always produce spotting in their progeny but may do so if conditions are favorable. Seedsmen have reported heavy hand-picking costs in areas where this disorder commonly occurs. Hand picking is not recommended as a method of control, but only for improving the appearance of the seed stock.—W. J. ZAUMEYER and B. L. WADE, Division of Fruit and Vegetable Crops and Diseases, Bureau of Plant Industry, United States Department of Agriculture, Washington, D. C.

Internal Breakdown of Pea Seed.—In the spring of 1934 dry seed of Laxtonian peas, *Pisum sativum* L., was received from Salinas, California. Of this seed about 20 per cent showed discolored areas surrounding the cen-

ters. The lesions are spherical in shape, irregular in outline, from 1 mm. to 5 mm. in diameter, and vary in color from a dull light brown to dark brown (Fig. 1). Attempted isolations of a suspected causal organism gave

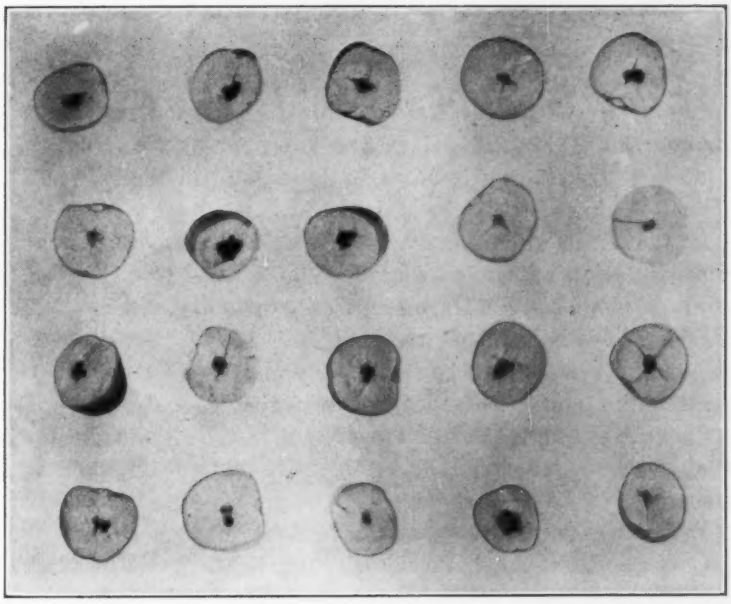

FIG. 1. Cross-sections of pea seeds showing internal breakdown.

negative results. From a germination test it was evident that the internally discolored seeds germinated as well as normal seeds from the same stock. The seed was harvested near Salinas in June, 1933, during relatively cool weather. The available records indicate that an excessive amount of water was supplied to the field during the ripening period.

This condition may be the same as that described by de Bruijn,[1] who explains it as the interaction between slow ripening and the occurrence of marsh areas in the field. She found among other things a decrease in sugar and soluble nitrogen and an increase in starch and insoluble nitrogen. De Bruijn's article covers the situation adequately but, inasmuch as it is not generally available to American readers, it was thought advisable to publish this brief note, so that seedsmen, pathologists, and other interested individuals would know of its occurrence in the Salinas region of California and be on the watch for it in other sections of the United States.

[1] Bruijn, H. L. G. de. Kwade harten van de erwten. Tidjschr. Plantenz. 39: 281–318. 1933. Krankhafte Erscheinungen ab Erbsensaat. Abst. in Die Ernährung der Pflanze 30: 68. 1934. [Abst. in Rev. Appl. Mycol. 13: 204–205. 1934.]

Seedsmen and growers have been somewhat apprehensive that the internal breakdown might be associated with some seed-borne disease. Since the affected seeds appear to be entirely normal externally, it is impossible to remove such seeds by hand picking.—B. L. WADE and W. J. ZAUMEYER, Division of Fruit and Vegetable Crops and Diseases, Bureau of Plant Industry, United States Department of Agriculture, Washington, D. C.

Leaf Smut of Rice in the United States.—Leaf smut of rice caused by *Entyloma oryzae* H. and P. Sydow, first described on rice from the Philippine Islands, is now known to occur in Japan and in the lower Mississippi Valley rice-growing areas in the United States.

Recently, S. Ito and his coworkers have cultured a fungus identical with *Entyloma oryzae* from leaf spots formerly attributed to a fungus known as *Sclerotium phyllachoroides* Hara and *Ectostroma oryzae* Sawada.

Specimens of this leaf spot received by the writer from E. Kawamura of Japan are identical with the small black leaf spot on rice in the United States. Examinations of these leaf spots on rice from Arkansas, Louisiana, and Texas have shown that there are present in the older lesions chlamydospores that, in all essential details, are identical with those given in the original description of *Entyloma oryzae* in Annales Mycologici 12: 197. April, 1914.—E. C. TULLIS. (Cooperative investigation between the Division of Cereal Crops and Diseases, Bureau of Plant Industry, United States Department of Agriculture, and the Arkansas, Louisiana, and Texas agricultural experiment stations.)

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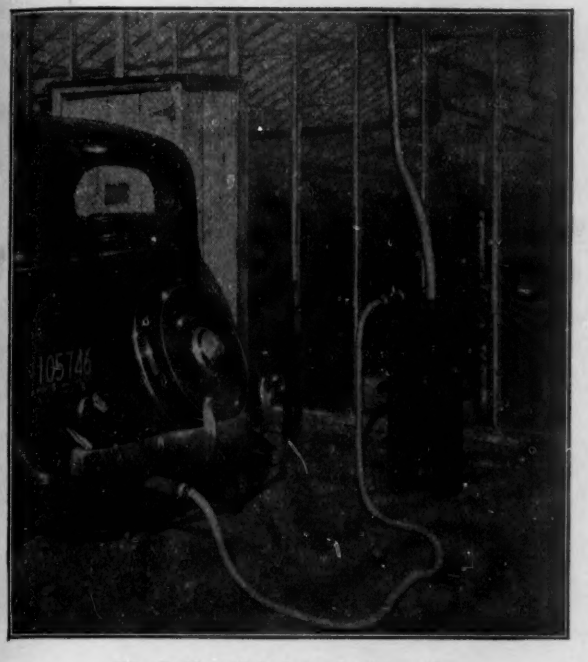

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